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VACCINE AND SERUM THERAPY

Including also a Study of Infections, Theories of Immunity, Opsonins and the Opsonic Index

BY

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PREFACE

Treatment of individual diseases with medicines or by methods having a selective curative action has until recently been limited. With the establishment of the germ theory of certain diseases and the development of information concerning immunity, new methods of specific treatment were made possible and are now practiced under the name of serum and vaccine therapy. As might be expected, the medical profession has been much interested in these methods of treatment, and applied them whenever possible. The development of vaccine and serum therapy has been slow, the methods have had to be revised and in some cases the results obtained have been found to be otherwise than was at first expected. Because of this, much confusion has arisen. The practitioner has not been able to keep pace with the developments and literature on these subjects, and finally has been forced to depend on the statements and recommendations coming from serum and vaccine laboratories, enthusiasts and even exploiters.

In this work an attempt has been made to state concisely and accurately the present knowledge concerning vaccines and immune sera. An effort has been made to establish theoretical and experimental evidence as well as clinical application of the specific treatment of bacterial diseases. To accomplish this, some space is given to infections in general, the theories of immunity, with especial emphasis on the opsonic theory of immunity, as well as the particular methods of vaccine and serum therapy.

Considerable space has been given to opsonins, the opsonic index and the importance of opsonins in health and disease. This has been done because since 1904 no subject has appeared more prominently or frequently in medical literature than that concerning opsonins, opsonic immunity, and bacterial vaccines. In the first presentations of discoveries on this form of immunity and specific treatment of bacterial diseases, great possibilities were promised. Methods, which would, according to Wright, give uniform success in treatment of the large class of bacterial infections and diseases, naturally received immediate and general attention by the medical world and were at once quite generally applied. This has been followed by much indiscriminate and unscientific use of these methods of specific treatment so that in the minds of

many, opsonins and vaccine therapy, have gone into disrepute as did tuberculin nearly twenty years ago. We now know tuberculin has many applications of importance in the diagnosis and treatment of tuberculosis, though this knowledge has been gained some years later than it would have been had it not been for improper exploitation. In the hope of avoiding a repetition of such an effect as far as opsonin and bacterial vaccines are concerned, this subject is given considerable attention. The opsonic index technique which is given here is the one taught the writer by Dr. W. G. Ross, who was for two years a pupil of Sir A. E. Wright.

An attempt has been made to bring the subjects taken up as nearly up to date as is possible. It is hoped that this work may furnish to the medical student and practitioner information which may lead him to a better understanding of the nature of infections and the subjects of immunity, and active and passive immunization.

Columbia, Missouri, April, 1909.

CHAPTER I.

INFECTIONS

By the term infection we understand the entrance of microparasitic living agents into the body tissue or substance, and the occurence of definite symptoms of disease as the result of the multiplication and action of the invading organisms. Infections do not always occur when pathogenic microörganisms are present in the body tissues, certain conditions being necessary for the establishment of a bacterial disease. These conditions may be broadly divided into two classes; those dependent upon the biological properties of the infecting organism, and those dependent upon the conditions in the host and tissues invaded.

Of the biological characters of importance in the infecting organism in the production of a bacterial disease, the most important are, that the microörganisms must be able to multiply rapidly and greatly in the tissues of the body, and that they must be able to produce poisons or substances harmful, either to some or to all, of the tissues of the body. The number of organisms necessary to cause disease varies with different species. Many more staphylococci are necessary to cause the formation of a furuncle than of anthrax bacilli to cause anthrax. The property to produce substances poisonous to the body tissues generally determines virulence. That virulence is of great importance is evident from the fact that innumerable so-called saprophytic bacteria which are present in the different parts of the body, grow and multiply there but because in their growth sufficient poison is not produced, cannot cause an infection. The virulence of an organism, according to numerous investigators, depends upon the presence of certain substances in the parasite which reduce the resistance of the body and its tissues. These substances are variously designated as lysins and aggressins.

Besides conditions in the causal organism, certain conditions must exist in the host or body so that infection can occur. Certain animals are insusceptible to the action of certain species of microörganism, thus for example, the horse and other domestic animals are naturally immune to venereal diseases. Again certain organisms can only produce disease when they are present in certain parts of the body and have entered the body by certain portals of entry. The spirillum cholerae can only produce its typical form of disease when it has gained entrance through the small intestine. Invasion of the dermis or epidermis by this organism produces no disease. The bacilli producing tuberculosis as well as certain other organisms, can cause infection when they have entered through the skin or through the mucous membrane. Certain organisms as Bact. diphtheriae, B. tuberculosis, and B. typhosus, show predilections for certain tissues but will also produce diseased conditions in other parts of the body.

In addition to the non-susceptibility of the host, the body possesses certain natural barriers to disease which must be overcome before infections can be produced. The unbroken skin usually offers a barrier to infection. Age, sex, race, occupation, etc., at times account for certain resistance to invasions of organisms. The resistance furthermore varies for the different organisms, as is evidenced by the fact that for B. tetanus a wound is necessary to produce lock-jaw, while for the glanders bacillus the slightest abrasion of the muccus membrane will furnish a focus for an infection. The body fluids, lymph glands, phagocytes, all offer resistance to infection. These barriers are overcome in various ways and under different conditions, so that while individuals may be immune at one time they may, at another time, be susceptible to the same infectious agent.

COURSE OF INFECTIONS.

From the foregoing it is evident that not only must the infectious organism come into contact with the body tissues to produce disease, but the organism must be able to grow, multiply, produce its poison, and overcome the resistance of a body susceptible to its action. The symptoms and signs of infections do not appear until a certain time after the invasion by the micro-

organism. This period is known as the "incubation period," and varies according to the biological characters of the infecting organism, but is also influenced by the number and virulence of the organisms and the individual susceptibility of the host.

The course of a disease is determined by conditions produced by the specific organism and partly by the distribution of these organisms. But even here it is to be noted that the course of the disease varies. Many of these variations we are by no means able to explain.

The symptoms and signs produced by an organism will vary as the organism acts locally or generally. In local infections the most marked disturbance occurs at the portal of entry of the microörganism, while in general infections the reaction manifests itself in all, or a large part, of the body. The infecting microörganism may produce disease either in a mechanical way because of large numbers, or, as is the case of most all infections, because of toxins which may act locally or be distributed over a large part or the whole, of the body. Some organisms, as the bacilli of diphtheria and of tetanus, produce extracellular toxins, while other organisms, as those causing typhoid fever and cholera, have intracellular toxins which are liberated supposedly when the organisms disintegrate.

After organisms have entered the body the distribution varies with the species. Thus we see that staphylococci form furuncles, carbuncles and pustules in localized areas, typhoid bacilli though causing lesions in the intestine are usually present in the blood stream, while the tetanus bacilli form a toxin which is distributed through the entire body. At times microörganisms do not remain localized at the portal of entry but pass on to the lymphatic glands and other parts of the body, here producing pathological conditions. In some infections the microörganisms gradually involve more and more of the tissues of the body. Microörganisms may first lodge in one part of the body and produce disease in that part and from these primary lesions, other parts of the body may become infected. When the blood stream is not only the carrier of microörganisms but becomes the place for growth and reproduction of the same, a septicaemia arises. While almost any of these conditions may be produced by any of the pathogenic bacteria, still certain organisms are more likely to produce one

or another of these conditions. Resultant of this tendency of microörganisms to produce particular kinds of conditions, the prognosis for infections varies with the species of microörganism producing the lesions or condition.

The same species of microörganism will not always be distributed in an identical manner; the location of the lesion and the condition produced varying with the properties of the particular strain in question and the particular part of the body infected. The appearance of general symptoms will vary greatly with the rapidity of the absorption of the toxin. Experimentally, intravenous and intraperitoneal injections of microorganisms are followed by symptoms earlier and more consistently than are subcutaneous injections.

The number of bacteria invading the tissues is of some importance. If few bacteria are present they may die without producing infections, while if larger numbers are present some of them will be likely to grow and produce disease.

At the point of infection, or portal of entry, the effects produced will vary with the infecting organism, some producing specific forms of inflammation along with those common to many bacteria. Even with the same species of microörganism the reaction will vary with the prevalence and virulence of the invading organism and with the anatomical structure of the part of the body involved. The local reaction, however, is not only produced when the bacteria enter the tissues of the body but also when the toxins produced by the microörganism are present in the tissues. The most commonly seen reaction at the portal of entry is that which results in pus formation, in which, due to chemotaxis, leucocytes accumulate. While a local reaction is usually produced at the portal of entry of bacteria, the reaction at times may be slight or absent entirely. A local reaction when produced is generally regarded as of importance in the protection of the body against the invasion of bacteria.

Besides the local reaction, general reactions usually follow in all severe infections. General reactions occur as a result of the action of toxins produced by the microörganisms when they are absorbed by body tissues. The general symptoms produced vary according to the location of the primary lesion, the extent of the process, the peculiarities of the organism, and the resistance

of body tissues. The more common general reactions are fever, digestive disturbances, effects on the nervous system, leucocytosis, anaemia, and enlargement of the spleen and other glands.

Infections and infectious diseases may be divided into two classes; one in which the disease runs a self-limited course, recovery coming after a definite period of time has elapsed, while in the other class the course may be extended over a very indefinite period of time.

The elimination of the casual or etiological factor of infections and infectious diseases occurs in various ways. When the seat of the disease is on the surface of the body, the organisms are eliminated with the diseased tissues, but when the organisms are found in parts of the body where this is impossible, the microörganisms must enter the general circulation, be allowed to pass through the glandular tissues and be eliminated with the secretions and excretions. In the blood and the body tissues, bacteria are in many cases destroyed by bacteriolytic and bactericidal substances. Sometimes after an apparent recovery from the disease, viable organisms may remain and at a later time again give rise to disease.

CHAPTER II.

IMMUNITY

Immunity may be defined as, non-susceptibility to a disease, or as the ability to resist the action of the causes of the disease. The body may be immune because of inherited properties or because it has become so during life. Immunity because of inherited properties is called "natural" immunity, while the immunity acquired during life is called "acquired" immunity.

Natural immunity is demonstrated by the non-susceptibility of the hen to the action of the tetanus bacillus. It is an immunity of race or species. This immunity at times may be reduced or removed by hunger, exhaustion, exposure to cold, etc. Certain closely related races or species of animals sometimes show a natural immunity and at times a natural susceptibility to the same infecting agent. This non-susceptibility is frequently called a natural resistance and at times is only an apparent immunity, depending in these cases on the common natural barrier to the entrance and development of disease producing organisms. Again, what may be regarded as a natural immunity is, in part at least, only a resistence to infection due to the inability of organisms to reach viable tissues. This is the case when the acidity of the stomach is sufficient to kill cholera organisms before they reach the epithelium of the intestine.

Acquired immunity only results after a pathological condition exists or has existed. The individual becomes immune, because he has survived a natural course of the disease, as is the case following an attack of scarlet fever; because he has gone through a modified form of the disease, as is the case in vaccination against small-pox; or, because he receives substances prepared by some other individual or animal that has gone through a natural

or modified course of the disease. Acquired immunity may be either active or passive.

Experimental active immunization is usually called vaccination and generally produces in the individual a modified form of the disease. All, or nearly all, of the symptoms are less severe than in the natural course of the disease. The individual in this case produces his own immunity. In artificial or intentional inoculation, the etiological factors, or more particularly, the causal organisms injected must be so modified that the natural course of the disease will not follow the inoculation or injection. Experimentally acquired active immunity is produced by the injection of living or killed microörganisms, or of toxins produced by these organisms. When living organisms are injected their virulence is usually reduced by passage through animals, growth at high temperatures, on artifical media, in the presence of chemicals, or growth in the presence or absence of oxygen, etc. Active immunity may also be acquired as a result of injection of living virulent organisms into such parts of the body where the disease will not be produced. The first amounts injected are usually smaller than those in subsequent injections. When bacteria are injected the immunity produced is a bacterial immunity and when toxins are injected a toxin immunity results.

In passively acquired immunity, the individual that becomes immune does little or nothing toward obtaining this immunity. It results from the injection of immunizing substances which have been prepared by an actively immunized individual or animal.

There are two classes of immunizing substances; those that act on bacteria, said to be anti-bacterial; and those that act on toxins, called anti-toxic. Of these two classes of immune substances, the anti-toxic has been more efficient in passive immunization.

After the formation of immunizing substances they do not remain indefinitely within the body, but are lost through the excretions either as immune bodies or as immune bodies broken down by the body cells. On this the difference in persistance of natural and acquired immunity is partly based, for in acquired immunity the supply of immune bodies is exhausted, while in natural immunity new substancss are constantly formed.

THEORIES OF IMMUNITY.

Acquired immunity, as has been stated, results because some individual or animal has gone through a natural or modified course of the disease. While acquiring immunity, the body and its tissues have in some way been modified. Various theories have been advanced to explain this phenomenon.

Klebs and Pasteur tried to explain the changes that occur in the acquisition of immunity by assuming that in going through a natural or modified course of disease, certain substances, necessary as food for the parasites, are used up. As the result of immunization, the food necessary for the microörganism is consumed and the individual is immune to a certain organism because this organism cannot obtain the food it needs for its existence and production of the disease.

Chauveau assumes that in immunization certain products of bacterial metabolism are retained in the body of the immunized animal or individual, which products protect the body tissues from further invasions by that particular parasite.

Metchnikoff, in 1883, formulated a theory according to which, during immunization, certain white blood cells and cells of certain organs acquire the ability to engulf and destroy the attenuated bacteria. This results in acquiring the ability to engulf and destroy more virulent forms of this same species of microörganisms.

EHRLICH'S SIDE CHAIN THEORY OF IMMUNITY.

In 1887 Salmon and Smith, Fóa and Bonome, Roux and Chamberland, and others found that immunity could be produced, not only by the injection of bacteria, but also, as a result of the injection of the products of bacterial metabolism. As a result, a chemical theory of immunity was advanced. According to this theory the tissues of the body are chemically changed by immunization.

Fodor, in 1887, was the first to observe that the normal body fluids, especially the blood, contain certain substances able to destroy bacteria. Buchner, Behring, and Nuttall, soon after Fodor's observation also recognized the bactericidal powers of certain sera. In 1889 Buchner reported that the cell-free blood serum contains certain substances which he called alexines. Alexines, he found, have the property of destroying bacteria.

In 1888 Hericourt and Richet, and in 1889 Babes and Lepp reported investigations which showed that by injections of blood serum from animals having acquired immunity to certain diseases, immunity to these same diseases can be conferred to other animals. Soon after this Behring and Kitasato reported successful immunization of rats to tetanus by means of injections of blood serum from rabbits immunized to tetanus. From the work of these investigators there developed the humeral theory of immunity.

Behring and Knorr found that the toxic product of the metabolism of the tetanus bacillus, could, without the presence of the bacillus, be used in immunization. The serum thus produced, they found would protect against the disease and the injection of the by-products of the tetanus bacillus. They assumed from this that toxin is neutralized by the immunizing substance in the immune serum, as a base is neutralized by an acid.

Numerous theories have been advanced to explain the facts observed and reported, but of all these theories one stands out prominently—Ehrlich's side chain or receptor theory, advanced by Ehrlich in 1897.

According to this theory, every living cell consists of a dominating nucleous (Leistungskern) and of side chains or receptors. The paradigm of this picture is to be found in the benzol ring with its side chains. The side chains or receptors of a cell are of many different kinds and serve usually to anchor and assimilate the food stuffs. At times, however, by means of the receptors the cells are combined with substances, not foods but cell poisons. The combination of the receptors of the cell with the receptors of foods differs from the combination of receptors of the cell and receptors of toxins. The combination of the receptors of the cell with the receptors of foods is so loose that after undergoing certain changes which are of value in assimilation of the food and the nourishment of the cell the food stuff is again eliminated without having injured the receptor. Toxins on the other hand, combine so firmly with the receptor of the cell that they cannot again be separated. As a result of this, receptors anchoring toxins are lost to the cell.

When a certain limit of anchoring of receptors by toxins has taken place the cell peri hes, and if a sufficient number of cells which have vital functions to perform are destroyed, death of

the individual follows When, however, the toxins do not anchor enough receptors or are not potent enough to destroy the cell and the organism, immunity results or follows.

In 1896, Weigert advanced the hypothesis that when tissue is lost the regeneration that follows is not only sufficient to restore the amount of tissue lost, but actually results in overproduction of this tissue. This process is observed in the over-production of segments in reptiles and of cell proliferation in granulating wounds in man and the animals. Ehrlich assumes that when the toxins combine with the receptors of the cell, the receptors are lost to the cell and as a result the cell is stimulated to reproduce the destroyed receptors. The process does not stop, however, when all lost receptors have been reproduced, for more receptors will be produced than were anchored by the toxins. The excessive production of receptors as a result of the stimulus following the anchorage of the receptors by toxins, Ehrlich assumes, leads to a disturbance of the equilibrium of the cell. As a result of this, the surplus receptors are thrown off and constitute what is known as the anti-body. This process can be actually observed in the lower animals in which extra parts, produced as a result of destruction of some parts, are cast off or lost.

The principal function of all receptors and side chains is to provide for the nutrition and metabolism of the cells. Receptors, and hence immune bodies, however, are not all of the same composition or even of the same general structure. In order to explain the different functions and actions of different immune bodies, Ehrlich has assumed that the receptors may be of simple constitution and structure or they may be complex. Any cell of the body may have large numbers of the same and different kinds of receptors. Ehrlich divides this large number of receptors into three orders.

RECEPTORS OF THE FIRST ORDER.

These are receptors that are of relatively simple constitution and structure and combine with substances that can be easily and readily anchored. Bacterial toxins anchor receptors of this order. The receptor here consists of only one haptophore, or combining group, which combines directly with the haptophore group of the bacterial toxin. This order of receptors and

immune bodies is demonstrated in Figure 1, and represents the type of receptor on which is based the action of bacterial toxin and the formation of anti-toxin.

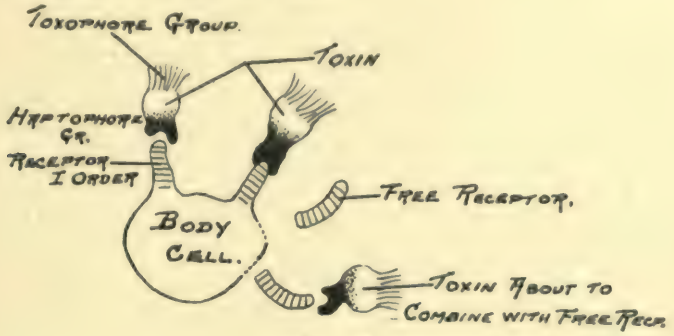

Fig. 1.

RECEPTORS OF THE SECOND ORDER.

The receptors of the second order are distinguished from those of the first order in that the receptors here have in addition to the haptophore group, a zymophore group. This zymophore group acts on the larger food particles, making them more readily assimilable. In a similar manner it acts on the bacterial cell. Receptors of this kind, possessing haptophore and zymophore groups, are broken off from the cells and circulate in the blood as agglutinins and precipitins after immunization by the injection of certain bacteria. The haptophore group of the immune body in an agglutinating or precipitating serum combines with the bacterial cells. The zymophore group, however, does not combine with anything but exerts its influences entirely through the haptophore group. The zymophore group is destroyed by age, acids, heating to 65° C., etc. Receptors and immune bodies of the second order are represented in Figure 2.

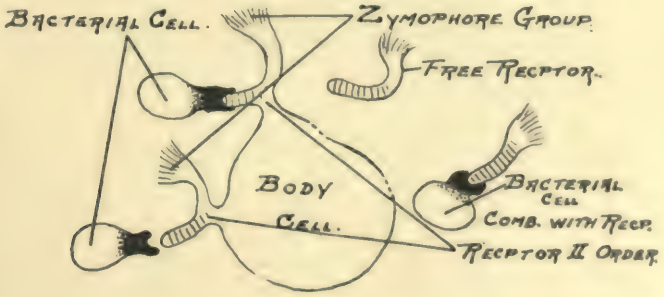

Fig. 2.

RECEPTORS OF THE THIRD ORDER

The receptors of the third order are likewise adapted to the anchorage of bacteria. These receptors, however, have two combining groups: one for the anchorage of cells or food substances and the other for the anchorage of substances which, acting through the receptors, can produce a ferment-like action. This latter substance is called an activating substance and is present in normal sera. It is known either as complement or alexin.

Complement is easily destroyed by ageing, acids, heat, 55° C., being sufficient to destroy it. When the receptors of this order are anchored by bacteria in numbers not sufficient to kill the cell but to stimulate it to over-production of receptors, the extra receptors are thrown off. The receptors thrown off constitute the immune body, amboceptor or substance sensibilitrice of Bordet. They have two combining or haptophore groups, one for the combination with bacterial or other cells and substances, called the "cytophylic" group; the other for combination with alexin or complement, called the "complementophore" group. It is by means of complement that the amboceptor is able to dissolve bacteria, red blood cells and other substances. Immune sera containing receptors of the third order are bacteriolytic, haemolytic, or cytolytic, depending upon whether they, together with complement, can dissolve bacteria, red blood cells, or other cells. The receptors of the third order are graphically represented in Figure 3.

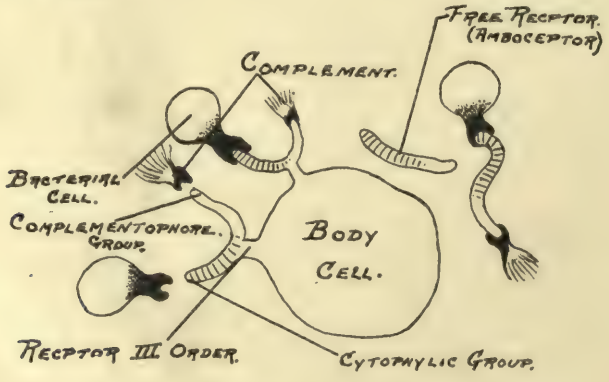

Fig. 3.

It will be observed that receptors of the first, second or third order can be produced in excess to form immune body or anti-

body. Specific immune bodies are produced for many substances, toxins, bacterial cells, red blood cells, ferments, etc. As a result of this multiplicity of substances that can cause the cells to produce anti-bodies, anti-bodies may be anti-toxic, agglutinating, precipitating, lytic, etc. To produce agglutination, precipitation or lysis it is necessary that a ferment-like substance be a part of, or able to combine with, the immune body. This ferment-like substance, as stated before, is labile and lost on ageing, heating, etc. When this is lost the serum containing the immune body is said to be inactive. In normal serum there is present a substance called complement which can again reactivate a lytic serum. The agglutinating power, however, cannot be restored by the addition of fresh complement. Complement can be preserved for long periods of time if the serum is dried without heat soon after the blood is drawn. Whether there is only one complement or a multiplicity of the same has not been definitely decided, Ehrlich assuming that there is a multiplicity of the same, while Bordet and Buchner claim there is only one.

It has been demonstrated that anti-toxic substances are important in preventing the action of toxins on the body cells, while lytic substances have been found to protect the body by the solution and destruction of bacteria. Whether the agglutinating or precipitating substances are of importance in destroying bacteria, is by no means certain. Numerous investigators assume that they injure and change the bacterial cells or substances used in immunization, while others claim that they exert no such action. It has been quite definitely observed that it is possible to cultivate and grow bacteria that have undergone agglutination.

As has been stated earlier, immune bodies are formed only for such substances as are able to combine firmly with the receptors of the cells, and it is on this assumption, that Ehrlich explains the impossibility of producing immunity to certain poisons, as the alkaloids, strychnine and morphine.

The cells whose receptors anchor the substances and cells for which immune substances are formed, are probably widely distributed throughout the body. The particular tissues in which anti-bodies are formed has not been determined and probably varies for the different substances to which immunization can be

obtained. It seems quite definite that in certain cases, internal organs may contain more immune substances than the blood serum. After immune bodies have been formed they do not remain permanently in the body, but are gradually lost through destruction in the body or lost with the excretions.

Ehrlich's views on the formation of immune bodies are quite generally accepted. His views, however, concerning the combination of toxin and anti-toxin, agglutinable substances and agglutinins, and substances capable of solution and lysins are not as universally accepted. Ehrlich assumes that the union of immune bodies and the specific substances is a chemical combination. Bordet assumes that in lytic sera the immune body acts as a mordant which sensitizes the substance capable of solution to the action of the complement. Field has explained the interaction of immune bodies and specific substance according to the principles of colloid chemistry, so that for example in a toxin and anti-toxin combination one is adsorbed (physical rather than chemical) by the other.

OPSONIN THEORY OF IMMUNITY.

It has been stated earlier that in 1883, Metchnikoff advanced his phagocytic theory of immunity. According to this theory the presence or absence of immunity depends upon the ability of phagocytes to engulf and destroy bacteria. Metchnikoff had given serum little consideration in immunity although he believed that some sera contain substances which stimulate the leucocytes to engulf bacteria. These substances he called "stimulins." The phagocytic theory, however, was practically replaced by the humeral theory of immunity because Fudor, Buchner, Behring, Nuttall and others had found that serum containing no cells whatever can destroy bacteria by solution. It was also found that antitoxin, which combines with toxin, is carried in serum.

From 1887 on the humeral theory of immunity gained a strong foothold and it was not until 1895 that attention was again called to the function of the leucocytes in immunity. In this year Denys and LeClef reported experiments from which they concluded that in immunization certian changes are produced in the serum, which make it possible for the leucocytes to engulf bacteria. In their conclusions they state that leucocytes from a

normal rabbit on the addition of normal rabbit serum, have only slight phagocytic action for the streptococcus. If, however, leucocytes from a normal rabbit, or from a streptococcus-immune rabbit are added to the serum from an animal vaccinated with streptococci, the leucocytes actively engulf and destroy the streptococci. When streptococci, able to produce erysipelas in a normal rabbit, are injected under the skin of a rabbit vaccinated against streptococci, disease is not produced. This protection is due, according to these investigators, especially to the ability of the leucocytes to destroy streptococci. They attribute the increased phagocytosis to an action of the immune serum on the leucocytes.

The results obtained and conclusions arrived at by Denys and LeClef were repeated by Bordet, who was not able to verify the results obtained by these investigators. Mennes found that immunity to Mic. pneumoniae depends on the action of serum which produces active phagocytosis but was not certain that this action is due to stimulation of the leucocytes.

In 1903 Wright and Douglas pointed out that there are certain substances in serum that so affect bacteria that they are more easily taken up and disposed of by the leucocytes. This substance they called "opsonin." They found opsonin present in normal and immune serum and from their investigations decided that the amount present in serum is variable, and can be increased or decreased by the injection of killed cultures of bacteria. To determine the amount of opsonin present in blood they modified the method of estimating phagocytic power introduced by Leishman in 1902. According to their method the average number of bacteria taken up by leucocytes, when bacteria, leucocytes and serum are mixed together and allowed to remain together for a certain time, is determined. The ratio of the average number of bacteria per leucocyte when patient's serum and serum from a healthy individual determine the phagocytosis, they called the opsonic index. The methods employed in the determination of this opsonic index will be taken up later.

In 1904 Neufeld and Rimpau, entirely independently of Wright and Douglas referred to the two well-known elements, antitoxin and bactericidal substances, in immune serum and stated that in anti-streptococcic and anti-pneumococcic serum they had

found a third factor of importance in immunity. This element, according to these investigators, sensitizes the corresponding bacteria and so modifies them that they are more readily engulfed by the leucocytes. This third factor in immunity they called "bacteriotropin." It is to be noticed that Neufeld and Rimpau worked with virulent streptococci and pneumococci. These organisms are not ingested by the leucocytes in a mixture of leucocytes, virulent streptococci or pneumococci and normal serum. However, when, instead of normal serum, a specific anti-serum takes part in the mixture there is ingestion even of cultures of virulent strains of these organisms. They furthermore showed that the serum does not act on the leucocytes but exerts its influence on the bacteria, sensitizing them to the action of leucocytes.

There seems now to be but little doubt that opsonin and bacteriotropin are the same substances, the greatest difference that can be assigned, being that bacteriotropins are probably what Wright and Douglas have called "immune opsonins."

Most investigators now take an intermediate position on the phagocytic and humeral theories of antibacterial immunity. It is quite generally accepted that both serum and leucocytes contain substances, which, acting after the manner of ferments, are able to dissolve bacteria. Just as all important manifestations of life are found in the normal and pathological cellular elements, so also the means of defense against harmful agents is probably closely related to the condition and functions of cells which prepare and secrete the protective substances by means of which bacteria and other harmful agents are destroyed or neutralized.

CHAPTER III.

THE OPSONIC INDEX

From the foregoing chapter it is seen that since the work of Denys and LeClef in 1895, it has been known that serum is of importance in phagocytosis. Little attention, however, was given to the various observations on this subject until the work of Wright and Douglas was presented. Although Neufeld and Rimpau discovered probably the same substances as Wright and Douglas, these investigators are seldom referred to in the discusion of the substances that change bacteria so as to prepare them for ingestion by leucocytes. There are two definite scientific reasons for the great prominence given to the work of Wright and Douglas: one is that they advanced and improved a technique for the determination of the amount of phagocytosis, by means of which the opsonic index could be determined; the other is to be found in the widespread interest in the methods they advanced for active immunization of patients by the injection of killed cultures.

In 1902 Leishman presented a method for the quantitative determination of phagocytosis. He mixed equal quantities of patient's blood and bacterial emulsion, which he then incubated for a time in a moist chamber. After incubation he made cover glass spreads which he dried, fixed and stained. On these slides he counted the number of bacteria ingested by the leucocytes, from which the average number per leucocyte was determined. This average he compared with the average per leucocyte obtained when normal blood instead of patient's was added to the bacterial emulsion. Leishman did not take into consideration the action of serum on bacteria or leucocytes but devised merely a "method of estimating phagocytic power."

Wright and Douglas, in their work on the determination of the opsonic index modified Leishman's method to meet their theory on phagocytosis. According to their observation opsonin

is a substance present in all serum. The amount varies in the different sera and as the amount varies so also will the amount of phagocytosis vary. To determine the phagocytic power it is necessary to have the three factors of phagocytosis in the mixture. These three factors are found in the blood serum, leucocytes and bacteria. A mixture of these three must be allowed to remain together for a definite period of time, after which the average number of bacteria taken up by the polymorphonuclear neutrophiles must be determined. This average number of bacteria per leucocyte is called the "phagocytic index." The phagocytic index obtained when serum from the patient is mixed and incubated with leucocytes and bacteria, is compared with the phagocytic index obtained when serum from a healthy individual is added to and incubated with a similar amount of the same emulsions of bacteria and leucocytes. The result obtained is called the "opsonic index."

The technique here given is essentially the one developed by Wright and Douglas and demonstrated in New York City in 1906 by Wright and taught by his associate, Dr. Ross. It is given somewhat in detail inasmuch as it is the one most generally used, even though many modifications have been suggested and followed by different investigators.

SERUM.

Blood from the individual whose serum is to be used in the determinations of the opsonic index, is collected as follows: A small glass capsule with one curved capillary limb is made as is indicated in Fig. 4. This tube is brought to a needle's point at "b" by heating over the pilot flame of a Bunsen burner. This

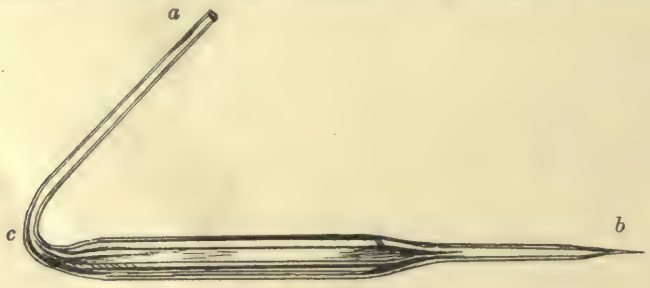

FIG. 4.

point is later used to puncture the finger or lobe of the ear. The end "a" of the capsule is left open. One of the fingers of the

hand is cleaned with alcohol and water, after which a bandage, handkerchief or rubber band is quite firmly tied around this finger. The windings of the bandage, handkerchief or rubber band are started at the base of the finger and run gradually toward the tip of the finger as more turns are put on. In this way there is produced an accummulation of blood in the veins and capillaries. Now, with the pointed end of the capillary bulb, the finger is pricked at a point about a quarter of an inch back of the nail, the needle end is broken off enough to open the capillary end. The open end "a" is now held close to the drop of blood which gradually fills the bulb; this is indicated in Fig. 5.

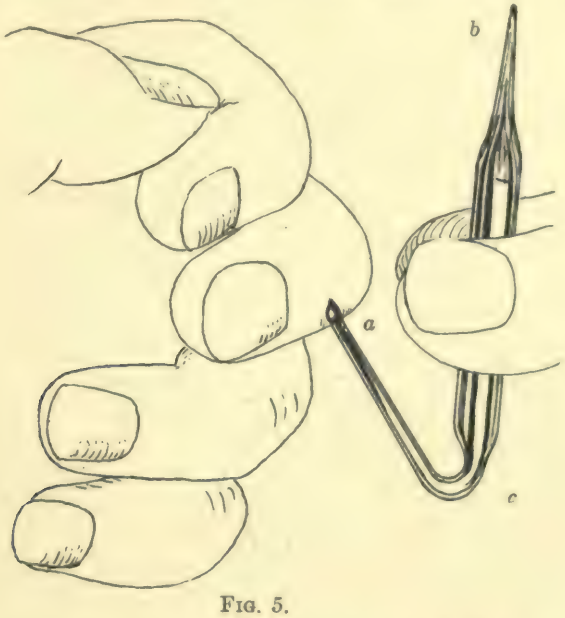

FIG. 5.

When enough blood has been drawn and collected in the bulb, the bulb is held between the fingers at "a" and "c" and the end "b" is heated and sealed off. As the end "b" cools the blood will draw away from the end "a" and finally all the blood will collect in the bulb proper. After this the bulb is again taken hold of at the curve "c" and by a rapid swing of the arm, as is practiced in shaking down a clinical thermometer, the blood is thrown down into the sealed end "b" as is indicated in Fig. 6. The blood is now allowed to clot in order to expel the serum. The serum may also be separated by hanging the bulb at "c" over the arm of the centrifuge, and centrifuging until the clot and serum have

well separated. The tube may now be opened by filing and breaking at "c."

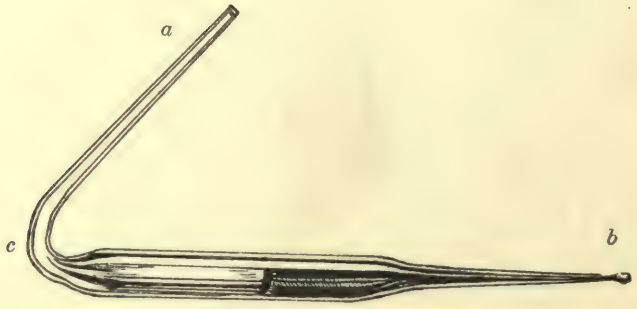

FIG. 6.

According to the original method of Wright and Douglas, blood was obtained from the patient and from a number of healthy individuals. Equal parts of the normal serum were taken and mixed together or "pooled." This was done so as to get a better normal serum. After it has once been established that serum from a certain healthy individual has an opsonic index of 1.0 for a particular organism when compared with pooled serum, this individual's serum replaces the pooled serum. After this has been established, the opsonic index of any patient for this organism is determined by dividing his phagocytic index by the phagocytic index of the "normal individual," as he may now be called.

LEUCOCYTES.

The leucocytes used in the determination of the opsonic index are usually obtained from the blood of supposedly healthy individuals, most frequently from the blood of the investigator himself. No particular stress, however, is laid upon the individual from whom the blood is obtained.

To obtain blood, preferably, the middle finger is cleaned, congested and punctured in the same manner as was followed in the collection of serum. Ten drops of blood are collected in about 10c.c. of a normal salt solution containing one per cent of sodium citrate. The sodium citrate is added to keep the blood from clotting. The tube containing the mixture is then centrifuged in a centrifuge of not too great speed. When the speed exceeds 1,200 to 1,500 revolutions per minute, the cells are too closely

packed together and form clumps. The mixture is centrifuged until the corpuscles are thrown to the bottom of the tube and the fluid above is clear, although it may be slightly straw colored. The supernatent fluid is then drawn off with a pipette. The corpuscles are washed free from serum and sodium citrate by again filling the tube with normal salt solution, mixing thoroughly and centrifuging until there again is a clear supernatent fluid. If the material in the centrifuge tube is now examined, one sees a clear fluid above and a red fluid in the lower part of the tube. The red fluid below, however, is not of the same shade throughout. The uppermost part consists of a grayish red layer, the leucocytes. These are layered above the red blood cells because of the difference in specific gravity of the leucocytes and red blood cells. It is from the gray layer that the leucocytes, to be used in the determination of the opsonic index, are obtained.

To obtain the leucocytes the clear fluid above is drawn off with a pipette and then with a clean pipette of about 1.6m.m. inside diameter, the leucocytes are removed. To do this the pipette firmly held in the hand, all of the air in the nipple is expelled, and then as the pressure on the nipple is gradually released, the open end of the pipette is held on the surface of the uppermost layer of grayish red color. This fluid will contain many red blood cells and also a relatively large number of leucocytes. After this fluid has been drawn into the larger part of the pipette the capillary end is sealed off. This fluid is called "leucocytic cream." Wright and Douglas state that in their experience there is no variation of the ability to engulf bacteria within the space of a few hours but that after three days the phagocytic power decreases to one-half or one-third of what it was when freshly drawn.

BACTERIAL EMULSION.

Inasmuch as Wright had early decided that opsonins are specific, such species or varities of bacteria are used in the determination of the opsonic index as may be of importance in the bacterial infection. In the selection of the particular culture to be used, there are two sources—either the different cultures isolated from the lesion are used or else the same species and varieties

of microörganisms are taken from a stock culture. Usually living freshly grown bacteria are used to make emulsions. The principle, or practically only exception to this has been in the determination of the opsonic index for the bacillus of tuberculosis, for which often killed and old cultures of the organism have been used to make the bacterial emulsion.

In all cases it is intended that the bacteria shall be well separated and suspended uniformly in salt solution. The technique for making the bacterial suspension varies with the different organisms, some of which it will be necessary to consider separately. The organisms for which the opsonic index is determined may be divided into three classes.

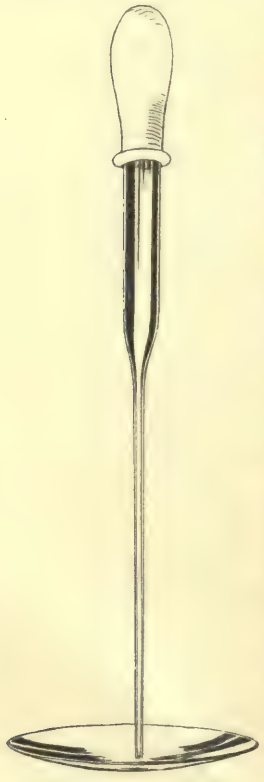

Fig. 7.

A. Many of the organisms belonging to this class grow on the ordinary media, while for others, media containing blood or other body fluids are necessary. The organisms occur singly or in pairs and when present in larger groups the cell aggregates can be easily broken up.

(a). Mic. pyogenes aureus, albus and citreus, B. coli, B. typhosus, B. pyocyaneus, etc., are grown on slant agar for twenty-four hours at 37° C. Various investigators, including Wright, have used four to five hour cultures of these organisms.

(b). Mic. gonorrhoeæ has usually been grown on hydrocele or human blood agar. Cole and Meakins obtained their cultures from growth on agar in which 0.5 c.c. of fresh blood had been added to 10 c.c. of ordinary agar. The age of the cultures used has varied from four to twenty-four hours incubation at 37° C.

(c). Mic. meningitidis, Mic. pneumoniæ, etc., produce good growth on sheep serum agar. The cultures have usually been incubated at 37° C. from twelve to twenty-four hours.

To each culture of the proper age about ten drops of sterile normal salt solution are added. With a platinum loop the cul-

ture is washed off the media and with a thick walled capillary pipette, with the end broken off squarely, the suspension is drawn into the pipette. The open end of the pipette is firmly pressed against a watch glass, as indicated in Fig. 7. When this is done only a small crevice will remain between the end of the pipette and the watch glass. The bulb is now compressed and the bacteria suspended in the salt solution are forced out through the small crevices. This is done to break up the small clumps of bacteria.

B. The organisms belonging to this class grow on the same kind of media as do those belonging to the first group. The organisms of this group form cell aggregates that are not easily broken up. The streptococci are the most important organisms in this class. Streptococci frequently grow in long chains, varying from two to thirty cocci. It is evident that a leucocyte may be able to engulf one or more cocci without being able to engulf a chain consisting of twenty or thirty cocci. The method for breaking up ordinary clumps will not suffice for breaking up chains of streptococci.

While observing the opsonic index for streptococci in erysipelas, the writer found it necessary to adopt some method to break up the long chains of streptococci frequently isolated from the lesions in erysipelas. This method consisted in the addition of 2 to 3 c.c. of sterile salt solution to each twenty-four hour culture on glycerine, glucose agar. After washing off the growth, the emulsion was put into a small test tube containing sterile sea sand; the tube was sealed in a flame and shaken for one and one-half hours in the shaking machine. The sand and emulsion in the tube were centrifuged for one minute and the supernatent fluid drawn off. In this way short chains, from two to four cocci, were obtained.

Another method was devised by Wright of Harvard University. This method differs only from the one of Wright and Douglas in the substitution of a paraffine block for the watch glass. When the open end of the pipette is held against the paraffine the crevices very small so that the chains are more completely broken up. This method gives very satisfactory results.

C. This class includes practically only one species of microörganisms, the bacillus of tuberculosis. The preparation of

suspensions of tubercle bacilli is attended by numerous difficulties, because this organism when grown on artificial media forms conglomerated masses. According to Wright and Douglas the living tubercle bacilli are heated to 100° C. before breaking up the clumps. Later Wright modified this techinque by heating the bacilli to 100° C. on three successive days. The clumps after this are ground up in an agate mortar or in a watch glass, two or three drops of 0.1 per cent salt solution being added at a time until two or three c. c. have been added. One-tenth per cent salt solution is used in making this suspension because with greater concentration the bacilli are again clumped. Later Wright added 1.5 per cent salt solution in making up the emulsion of tubercle bacilli because he found that this concentration is necessary to prevent spontaneous phagocytosis. When the heated bacilli are thoroughly rubbed and suspended in 1.5 per cent salt solution a homogeneous mixture, containing but few clumps and many isloated bacteria, results. This mixture is then centrifuged at high speed for about ten minutes. After this the supernatent fluid is drawn off, and enough salt solution added to get the right concentration of the emulsion.

While this method gives a fairly homogenous suspension of tubercle bacilli, still in an emulsion made in this way many of the tubercle bacilli are broken up. In determining the opsonic index it is necessary to count the number of bacilli taken up by the leucocytes, and when there is fragmentation of bacilli it is necessary either to count each fragment as one bacillus, or else to determine the fractional part of a bacillus. Either of these methods is most unsatisfactory.

Sellard and Jeans have emulsified the living tubercle bacilli in the same manner that has been used for the emulsification of other bacteria. After this they have killed the bacilli by exposing the emulsion to sunlight for a number of hours—ten hours being sufficient to kill all tubercle bacilli present. In such emulsions they have gotten no spontaneous clumping, no fragmentation, nor spontaneous phagocytosis.

Walker has recommended a method, according to which Dorsett's egg medium is heavily inoculated with an actively growing culture of the bacillus of tuberculosis. After fourteen to eighteen hours of incubation at 37° C., salt solution is squirted over the

culture, which is now rubbed off into the salt solution, the clumps being broken up with a platinum needle. The suspension is then filtered first through a loosely packed cotton filter and later through a filter made with scraped filter paper. Filtration is repeated until all clumps are removed, after which the clump-free suspension is heated to 75° C. for twenty to thirty minutes.

STRENGTH OF BACTERIAL EMULSION.

The strength of bacterial emulsion most commonly used has a slightly opalescent appearance. It has been found that cloudiness may be absent or be only very slight and still the emulsion may contain too many bacteria. According to Wright's instructions the strength of the emulsion to be used is one which gives an average count of from five to eight bacteria for leucocytes when normal serum, leucocytes and the suspension of bacteria are incubated for the proper length of time. Walker has obtained better results with heavier suspensions of bacteria and diluted serum. It is not very difficult to make an emulsion of staphylococci, for after a bit of experience it can be determined by the naked eye whether the suspension of this species is heavy enough or not. The tubercle bacillus, however, presents greater difficulties, it frequently being necessary to actually make a trial test for the bacterial emulsion. Simon and others have proposed the numerical determination of the number of bacteria per c. c. in the bacterial emulsion.

MIXTURE OF BACTERIA, LEUCOCYTES AND SERUM.

From the definition of the opsonic index, it is evident that similar quantities of the same factors must be taken to make comparative mixtures. Furthermore, according to the methods devised by Wright, equal amounts of each factor are mixed together. When it is desired to use diluted serum equal volumes may still be used if the serum be diluted properly before taking the volume.

In order to get equal volumes Wright has made a capillary pipette, the walls of the capillary part of which are thick. The end of the pipette is broken off squarely. About a quarter or one-half inch from the open capillary end a mark is made with a soft wax pencil. This pipette is shown in Fig. 8. By means of

a rubber teat which is attached to the large end of the pipette, leucocytic cream is drawn into the capillary end up to the mark. Then the capillary end is withdrawn from the leucocytic emulsion and the emulsion in the tube is drawn about one quarter inch further up into the tube. After this the capillary end of the pipette is immersed in the bacterial emulsion, which is taken into the tube up to the mark. The tube is withdrawn and again a small amount of air drawn into the capillary tube. After this the serum is drawn in, again taking the amount necessary to fill the capillary pipette to the mark. Walker has suggested that small quantities of serum and leucocytic cream be put into small tubes and that serum and leucocytes be taken only once from each of these small tubes. This is done in order to avoid carrying materials from one tube into another.

The capillary pipette now contains equal volumes of leucocytic cream, bacterial emulsion, and serum, as is shown in Fig. 9.

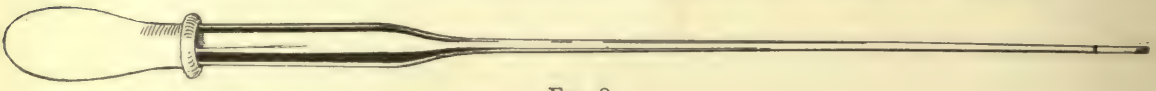

FIG. 8.

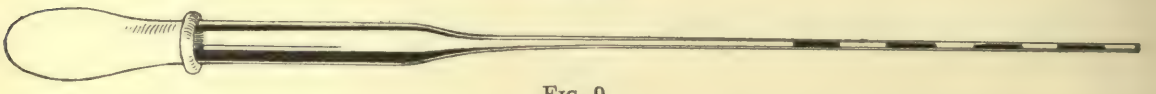

FIG. 9.

All three volumes are now forced out onto a glass slide, being drawn in and out with the pipette in order to mix the three parts thoroughly. When this has been done the mixture is drawn into the tube and allowed to come about half way up the capillary part when the open end is sealed off in a pilot flame of the Bunsen burner. Walker mixes the three factors in a small test tube taking precautions against air bubbles. Slides, however, have seemed better adapted especially because air bubbles are more easily avoided.

After sealing the capillary pipette, containing the mixture of leucocytes, bacteria and serum, it is incubated at 37° C. Realizing that the pipette containing the mixture will not assume the temperature of the thermostat if it is merely exposed to the air in the thermostat, Wright originated a so-called opsonizer.

The length of time for which the mixture is incubated varies for the different organisms. It is to be noted that up to a certain

limit the longer the period of incubation the more marked will be the phagocytosis. The incubation time must in some cases be limited because of solution and agglutination of bacteria. In all instances the mixture containing patient's serum and that containing normal serum must be incubated at the same temperature and for the same length of time. The time of incubation varies from five to thirty minutes, depending upon the species of microörganism and the properties of the blood serum tested.

After incubation the nipple is removed and the capillary end of the pipette is broken off, the nipple is then replaced and the leucocytes, bacteria and serum are again thoroughly mixed on a glass slide, after which smears are made.

SMEARS.

If reference again is made to the principle involved in the determination of the opsonic index, it will be noted that the index shows the ratio of the number of bacteria taken up by the polymorphonuclear neutrophiles when patient's serum is a part of the mixture, to the number of bacteria taken up by the same class of leucocytes when normal serum is a part of the mixture. It is therefore necessary to count the number of bacteria in the polynuclear leucocytes. In order to have an abundance of leucocytes the grayish red layer is taken off the centrifuged blood. This contains a relatively larger number of white blood cells than does blood freshly drawn. If a drop of this leucocytic cream is spread out by dropping a cover glass onto it, fixed, and stained, many red blood cells and some white blood cells will be found in the field of the microscope. It would, however, be a tedious task to find fifty or one hundred polynuclear neutrophiles. Wright and Douglas have devised an ingenious method to gather the leucocytes together in certain parts of the slide. The method of doing this is based on the difference in size of red blood cells, mononuclear and polynuclear leucocytes; the polynuclear and large mononuclear cells being largest in size.

The method of Wright and Douglas is as follows: A slide is cleaned and slightly scratched by rubbing with jeweler's emory paper to slightly roughen the surface of the glass slide. On the left end of this slide a drop of the incubated mixture, which has

been thoroughly mixed after incubation, is placed. Then a slide with a smooth edge is made into a spreader by breaking off a corner. This is done so that the two margins of the spread shall be on the slide instead of running over the edges of the same.

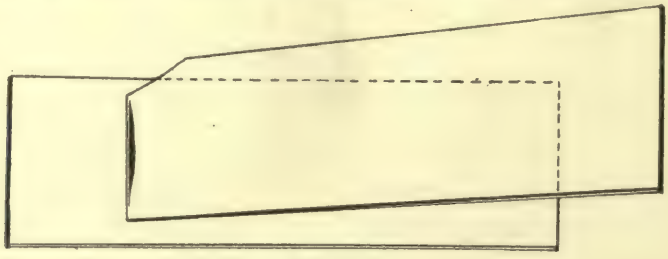

FIG. 10.

The narrowed end of the spreader is now touched to the slide and the drop of blood allowed to run to the under edges of the spreader. The spreader, held at an angle of about 35° to 45° is drawn over the slide as is indicated in Fig. 10. The spread on the slide will have an outline as is indicated in Fig. 11.

The different steps taken in making the spread are essential to secure good smears. When the spreader is pressed firmly enough against the slide while it is being drawn over the same,

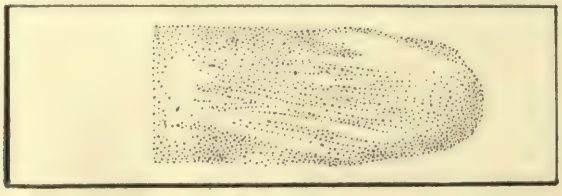

FIG. 11.

the polynuclear leucocytes, being larger than the red blood cells, will slide out at the edge of the spreader or be drawn to the end of the spread. Care must be taken not to make the smear too thin.

FIXING AND STAINING OF THE SMEARS.

After the smear has been made it must be fixed and stained for examination. Fixing and staining is usually accomplished by the ordinary blood stains, Leishman's, Wright's, Jenner's all giving good results,except when the index for the tubercle bacillus is to be determined. The writer, however, has gotten more sat-

isfactory results for organisms, other than the bacillus of tuberculosis, by fixing the spread with methyl alcohol for one minute, washing off with water and then staining with Loeffler's methylene blue for three to five minutes. In staining slides on which the index for the tubercle bacillus is to be obtained, a modification of Wright's method has given most satisfactory results. The smears are first fixed in a saturated solution of bichloride of mercury which is then washed off with water. After this the slide is immersed in a jar containing Ziehl's carbol-fuchsin. The stain is heated by placing the jar in a heated water bath. After five minutes of staining in hot carbol-fuchsin, the stain is washed off and the smear is decolorized in a mixture of 97 parts of alcohol and 3 parts of concentrated hydrochloric acid. The smear is counter stained in a solution of one-half grams each of sodium carbonate and methylene blue in 100 c. c. of water. This counter-stain acts rapidly and has the advantage that if the slide be over stained in the process it can be easily decolorized with warm water.

EXAMINATION OF SMEARS.

The spreads are examined by first going over the slide with the low power of the microscope to determine whether the slide has been properly stained and also to find the part of the slide where the polynuclear leucocytes are most numerous. Usually it is found that these are most abundant near the margins and at the end of the smear. Wright has recommended that those at the end of the smear be examined.

Under the high power of the microscope the number of bacteria engulfed by a definite number of polynuclear leucocytes is determined. The number of leucocytes examined varies. Wright based many of his determinations on examinations of twenty leucocytes. Most investigators, however, have counted the number of bacteria in fifty or more polynuclear leucocytes.

The average number of bacteria per leucocytes is spoken of as the phagocytic index.

CALCULATION OF THE OPSONIC INDEX.

The opsonic index of Wright is determined by dividing the phagocytic index obtained when patient's serum is used with

a certain leucocytic cream and bacterial suspension, by the phagocytic index obtained when serum from the healthy individual is used with the same leucocytic cream and bacterial emulsion. This may be illustrated in a concrete case as follows: If the average number of staphylococci phagocytosed as determined by counting the cocci in fifty leucocytes is eight when the patient serum is used, and ten when normal serum is used, then eight-tenths or 0.8 is the opsonic index.

CHAPTER IV.

CRITICISMS AND MODIFICATIONS OF WRIGHT'S OPSONIC INDEX DETERMINATIONS

Wright's publications on opsonins, the part they play in immunity and the method of determination of the opsonic index lead to much investigation on these subjects. Many investigators followed Wright's methods closely while some have followed what they **supposed** were Wright's methods. The first reports of work done by investigators other than Wright and his pupils, agree strikingly with the results obtained by Wright. Later results, however, were not as favorable either before or after Wright himself and some of his pupils had demonstrated and taught his technique. For a time want of personal skill and ability, improper methods of work, and lack of ability to manipulate were supposed to account for results which did not agree with those obtained by Wright. Numerous investigators, however, after having received instructions from Wright and his pupils on the methods of determining the opsonic index, seriously questioned the methods of Wright. This list of investigators includes Park, Simon, Baldwin, Cole, Moss, Potter, Bolduan, Walker, and many others. On the other hand there are numerous investigators who have not questioned the technique and the results obtained by following the methods of Wright. These investigators followed principally the opsonic index in the various bacterial diseases and made efforts to determine the nature of opsonins, their importance and behavior in the various diseases.

The opsonic index, according to Wright, is obtained when the average number of bacteria ingested per leucocyte in a mixture of patient's serum, bacterial emuslion and leucocytes incubated for a certain period of time, is divided by the average

number of bacteria ingested by the leucocytes in a mixture of the same quantity of serum from a normal individual, the same bacterial emulsion and leucocytic cream, incubated for the same length of time at the same temperature. In the process of the determination of the opsonic index mechanical technique, serum, bacterial emulsion, leucocytic cream, and calculation of the average number of bacteria ingested are of importance. Inasmuch as the results obtained by the different investigators are so discordant and inconsistent, all of these factors have been considered, emphasized and, in the minds of some at least, improved.

MECHANICAL TECHNIQUE.

The mechanical part of the technique of Wright has probably been less criticised than any other part of his method. Various investigators have reported their technique in full and some at least have shown that they have not interpreted Wright's explanations of the same correctly, while others have modified the technique in order to give greater accuracy to the method of the determination of the index.

Only some of the modifications suggested can be mentioned. Barber, thinking that the mark on the pipette as made by the wax pencil, is too wide and therefore leads to inaccuracies in the amounts used in the mixtures, has suggested that the mark on the capillary pipette be made with a glass hair dipped in Bismarck black. This suggestion, however, is not generally followed as it is possible for one experienced in handling the capillary pipette to draw fluids to the upper or lower edge of the wax pencil mark with as much accuracy as it is to take it to the mark made by the glass hair.

Walker has suggested the use of a water bath for the incubation of the mixture of serum, leucocytes, and bacterial emulsion. Undoubtedly this has advantages over incubation for short periods of time in the air in the thermostat. Wright's opsonizer, however, is more convenient and almost immediately brings the pipette to the temperature of the metal of the opsonizer.

For the collection of serum, Walker has suggested that the capsule be held in position by inserting it into a slit in a slide box. Unless one is taking his own blood, however, it is

about as convenient to hold the capsule in the hand as to hold the punctured finger near the table on which the slide box rests.

Walker has also advocated that small quantities of full strength or diluted sera and leucocytic cream be put into separate small test tubes in order to avoid carrying bacterial emulsion to the lencocytic cream from which other preparations are to be made later. This method is certainly to be recommended.

Walker has also modified Wright's methods concerning the capillary pipette containing the mixture. In making opsonic index determinations, Walker breaks off the large end of the capillary pipette and seals both ends of the capillary tube. When this is done it becomes necessary to again fit the capillary part of the pipette to a larger tube to which a rubber teat can be fastened. This Walker accomplishes by fastening a thin rubber sheet over the open end of the large pipette. Thisworks as a diaphragm and can be easily punctured by the capillary tube.

SERUM.

Immunity to and recovery from numerous infections according to Wright's theory is due to the presence of opsonin. Opsonin prepares bacteria for ingestion by the leucocytes and is a substance contained in the serum. In the determination of the opsonic index the ratio of bacteria ingested by the leucocytes is established by mixing bacteria and leucocytes in one case with patient's serum and in another case with serum from healthy individuals. It is thus evident that the serum is the important and valuable factor.

Normal Serum.

In the determination of the opsonic index numerous investigators have been struck by the apparently unexplainable indices found. This has lead to the investigation of the opsonic index obtained with serum from the normal individual, to determine whether the index does not vary in the normal, as well as in the infected, individual.

If reference is made to the technique, as proposed to obtain the normal phagocytic index, it will be observed that Wright recognized that there is a difference in the amount of opsonin present in serum from the normal individual. He states that

the opsonic index of a normal individual will vary from 0.8 to 1.2. To obtain the true amount of opsonin in normal serum, he pools equal quantities of sera from several normal individuals. However, after it has been established that a healthy individual has an opsonic index of 1.0, as determined by estimation with pooled normal sera, he uses serum from this individual as a normal serum. Wright's own determinations, from which he concedes that there are variations in the amount of opsonin present in the blood of healthy individuals, discredit the accuracy either of the method of determining the opsonic index or the value of determining the same. If the opsonic indices of two normal individuals is 0.8 and 1.2 respectively on the same day as determined by co parison with pooled normal serum, then as compared to each other their opsonic indices vary from 0.66 to 1.5 respectively. It is thus evident that from Wright's concession the index of a normal individual may vary from 0.66 to 1.5 when one serum is used as a control for the other.

Indices for normal serum as actually determined will not even vary within these limits. The writer sometime ago reported results on a series of ten specimens of blood from ten healthy adults, studied to determine the differences in opsonic power in healthy individuals. The leucocytes were washed twice with 0.85 salt solution. The number of staphylococci taken up by 100 polynuclear leucocytes was counted in two preparations, separately incubated. The phagocytic indices as determined by two workers A and B are as follows:

Source.	A	B
N	9.48	7.63
J	9.16	5.20
B	8.75	6.80
McL	7.27	4.37
Z	6.63	5.12
C	6.60	5.37
S	5.89	4.82
O	5.84	4.57
H	5.49	6.03
Ns	4.91	4.37

The great error introduced by this method becomes evident upon the determination of the opsonic indices when each serum is used for comparison with all of the others. In the series A, the normal opsonic index varies from 0.52 to 1.93, while in series B it varies from 0.57 to 1.75. Moreover, the indices obtained by the two workers A and B vary considerably for the same sera.

Bolduan has reported determinations of the opsonic index of normal individuals and finds that as far as the bacillus of tuberculosis, staphylococci and streptococci are concerned, there is marked variation not only in different individuals but also from day to day.

Moss has found opsonic indices for staphylococci to vary from 0.18 to 0.56 in normal rabbits.

The same serum has also been tested a number of times with the same leucocytes and bacterial emulsion. Bolduan reports opsonic index determinations for a single serum which vary from 1.05 to 1.46. The writer has reported determinations made by taking five specimens of blood from the five fingers of the same hand of a healthy adult. The leucocytes used for the determination were washed twice in 1.5 per cent potassium citrate in normal salt solution. The phagocytic indices for Mic. pyogenes aureus are shown in the table:

	1st 100 leukocytes counted.	2d 100 leukocytes counted.	Phagocytic index.
Specimen 1	166	196	1.810
" 2	176	183	1.795
" 3	198	199	1.985
" 4	185	175	1.800
" 5	230	190	2.100

If the indices for each specimen of blood be compared with all the others, the opsonic index as determined by counting the number of cocci in two hundred cells will be found to vary from 0.85 to 1.22.

It thus seems evident that if normal serum varies as much as has been found, the variations from the normal, unless marked, can indicate but little. The most that would seem warranted is to determine whether a serum is "high" or "low" in opsonic content, as the case may be.

Dilutions of Serum.

Wright has usually used undiluted serum in the determination of the opsonic index. Simon, Lamar and Bispham have shown that by the dilution of some sera there often is a rapid exhaustion of phagocytic power of the leucocytes. They have observed that undiluted pig's serum manifests a most intense opsonizing effect on staphylococci but that this action diminishes markedly upon dilution of the serum. Human serum, on the other hand, though not having as marked an initial opsonizing

power, will retain this power on much greater dilution. They have further found that in human beings, who are supposedly in good health, the phagocytic power in concentrated serum will, in some cases, diminish in proportion to the degree of dilution, while with the serum of other individuals, more rapid exhaustion takes place.

Moss has compared the opsonic index in various dilutions of serum and has found that the index obtained in one dilution is not proportional to that obtained in all the other dilutions.

Walker's reports on the results obtained by the dilution of serum differ from those obtained by Moss. Walker has found that with many bacteria, undiluted normal serum will opsonize so many bacteria in a heavy suspension of bacteria that the leucocytes will contain so many microörganisms, that counting is impossible. If the bacterial suspension be made less heavy fewer bacteria will be taken up by the leucocytes, but under this condition the serum will not be exhausted of its opsonin and part will be lost in the estimation of the opsonic index. A serum containing much less opsonin may opsonize just as many bacteria in a light bacterial suspension as a serum containing more opsonin. "When thin suspensions of bacteria for which much opsonin exists in the serum are used it generally happens that both the sera sensitize all the bacteria so that the work if accurately done will produce equal phagocytic indices for both—in other terms, an opsonic index of unity—regardless of the real relation of the serum. Phagocytic indices proportional to the sera tested may readily be obtained by diluting all the sera equally to a sufficient degree, and using with these diluted sera a thick bacterial suspension." For some bacteria as B. typhosus, certain strains of streptococci and tubercle bacilli, Walker does not recommend the dilution of serum, while for staphylococci, some strains of streptococci and tubercle bacilli and for B. coli he does.

Walker's views concerning the utilization of all the opsonin capable of sensitizing the particular species of bacteria by the use of heavy bacterial suspension and diluted sera, must be favorably received. Before they can be accepted and followed it will, however, be necessary to disprove the results of Moss and others concerning the dilution of opsonin by the dilution of the serum.

BACTERIAL EMULSIONS.

Wright, in his technique, attempts in every case to break up all clumps of bacteria and tries to get such strength of suspension that the number of bacteria per leucocyte can be easily determined. With certain organisms trial tests are necessary. After this, the suspension may be diluted or bacteria added as may be indicated.

Most investigators have experienced difficulty in obtaining suitable suspensions and Wright himself has changed his technique for making bacterial suspensions from time to time. The difficulties that have been experienced are of two kinds: those dealing with the strength of the bacterial suspension, and those concerning the condition of the microörganisms in the suspension.

Difficulties depending upon the strength of the bacterial suspension. Numerous investigators have given definite figures to be obtained for the phagocytic index with normal serum. In most cases it is desirable that the phagocytic index for normal serum be between six and fifteen. Simon has found that when the emulsion contains between 666,000 and 2,000,000 microörganisms per cubic millemeter the best results are obtained. Recently Walker has emphasized certain facts that must be taken into consideration in determining the strength of the bacterial emulsion. If the suspension does not contain enough bacteria to exhaust all the opsonin in the strongest serum, a certain amount of opsonin will, according to Walker, be lost in the estimation of the opsonic index. In order to get the right strength of bacterial suspension, Walker tests the same with serum diluted 1 to 30 and 1 to 15. If the 1 to 15 dilution of serum shows a phagocytic index twice as great as that obtained in the 1 to 30 dilution then enough bacteria are present in the suspension. Too such a suspension he, however, adds more of the culture, because having more bacteria than are absolutely necessary will not change the opsonic index but will certainly furnish enough bacteria to exhaust the serum of its opsonin. Only with the staphylococci will a too heavy suspension effect the true index. This Walker believes is due to the action of a product of bacterial growth. To overcome the excessive phagocytosis when heavy suspensions of bacteria are used, Walker dilutes serum. However, as has been

mentioned before, there are conflicting views concerning the effect of dilution of serum for which the opsonic index is to be determined.

DIFFICULTIES ARISING BECAUSE OF THE PARTICULAR SPECIES OF BACTERIA IN THE SUSPENSION.

Anyone who has made opsonic index determinations has at times had slides to examine in which the bacteria were not well stained and in which the microörganisms were apparently clumped. Frequently only shells of organisms remain and at times the bacteria are completely dissolved. These difficulties are apparently dependent upon the particular culture used but to a greater extent upon the agglutinins and lysins present in different sera. To overcome this difficulty young cultures are preferred because they stain better than do old cultures. To overcome agglutination and lysis the serum is in some cases heated to 55° or 65°. The clumping of bacteria in opsonic index preparations is given little attention by Wright. Other investigators have, however, regarded clumping of bacteria as responsible for the occasional enormous differences of the indices determined. Walker gives tables which indicate the objection of clumps on the uniformity of phagocytosis and has prepared a technique by which he obtains clump-free bacterial suspensions. According to this technique the bacteria are rubbed gently in a mortar with small, and gradually increasing, quantities of salt solution until a thick suspension is obtained. This suspension is then filtered through moistened scraped filter paper until in a stained specimen no more clumps are found. Even though a technique may be devised to obtain relatively clump-free suspensions of bacteria, this will not do away with the clumping occasionally observed in opsonic index preparations, because the action of agglutinins in undiluted or only slightly diluted sera will again cause clumping of the bacteria.

LEUCOCYTES.

There has been but little criticism of the method of collection of leucocytes, as proposed by Wright. Simon has used 0.1 per cent ammonium oxalate instead of 1.0 per cent sodium citrate in salt solution. Some investigators have drawn the corpuscles

off after the first centrifugalization while others have attempted to remove the serum of the blood and the citrate or oxalate solution by several washings with normal salt solution.

To the source of the leucocytes Wright attaches little importance, while most other investigators have aimed to obtain leucocytes from the blood of apparently healthy individuals. The work of Peterson and Hiss and Zinsser indicates that the leucocytes contain substances that are of importance in immunity and that these substances vary in the leucocytes of normal and immunized animals. For this and other reasons it seems advisable in all cases to obtain the leucocytes from the blood of normal individuals.

Walker draws the blood from which the leucocytes are to be obtained into citrate solution heated to 37° C. The leucocytic cream is kept at this temperature up to the time of mixture with serum and bacterial emulsion. He believes this to be of great importance. In experiments performed by the writer and others, this has not been found to be of especial value in the production of uniform phagocytosis, nor do Walker's tables show a marked uniformity, for the number of bacteria per leucocyte vary from 1 to 17 in twenty-five leucocytes examined on the same slide.

CALCULATION AND DETERMINATION OF THE OPSONIC INDEX.

According to Wright's method the opsonic index is determined and calculated by dividing the degree of phagocytosis obtained with patient's serum by that obtained with serum from a normal individual.

Simon has proposed an entirely different method for the calculation of the opsonic index. He had observed that with many species of bacteria, due to lysis and fragmentation, great difficulty is experienced in determining the phagocytic index and that the strength of emulsion greatly changes the amount of phagocytosis. To remedy this, Simon first proposed that the percentage of phagocytic cells be determined by counting 50 cells taken in sequence. Later Simon compared the percentage of phagocyting leucocytes with patient's serum to the percentage of phagocyting cells with pooled normal serum. The value ob-

tained for pooled normal serum he called 1.0, and the index determined in this way he called the percentage index as contrasted to Wright's bacillary index. These two indices Simon finds can be made to agree to the second decimal place.

Moss and others have found that the absolute numbers of cells phagocytosing depends in normal or slightly diluted sera on the strength of the bacterial suspension, and that in low dilutions practically all the cells can be made to engulf bacteria. It is evident that by Simon's method only sera having low indices can be investigated, unless the bacterial emulsion or serum are diluted. Simon has recommended that light bacterial suspensions and at times diluted sera be used so as to be able to determine the percentage index.

Strong, in his early experiments with anti-plague serum, determined the index according to Wright's method. Later, however, he substituted this method by one in which the highest dilution of an immune serum which gives a marked phagocytosis is compared to the same dilution of a normal serum. If, with a certain dilution of an immune serum marked phagocytosis is observed while the same dilution of a normal serum gives only slight phagocytosis, the immune serum is regarded as having an increased opsonin content. Even on this method Strong places little reliance.

Although Simon prefers to accept his index to Wright's when there is disagreement between the two, most investigators have preferred Wright's opsonic index.

LEUCOCYTES IN WHICH THE BACTERIA ARE TO BE FOUND.

According to Wright's method the bacteria are to be counted in the polynuclear neutrophiles. These cells when Wright's technique is followed will be found along the margins and end of the spread.

In all work on the determination of opsonic indices it has been found that there are great differences in the number of bacteria taken up by the individual leucocytes. Moreover, great differences have been found in the phagocytic index as determined by different investigators examining the same slide. Even the same

investigators have obtained markedly different phagocytic indices on the same slide. The writer has reported work showing that there is not always agreement between results obtained by counting the bacteria in 100 and 200 polynuclear leucocytes on the same slide. Bolduan, Moss, Cole, Jeans and Sellards, Simon and others have published results showing striking differences in the counts obtained in 50, 100, 200 and more cells. Bolduan supposes that counting the number of bacteria ingested by 100 or 150 cells will give a fairly correct average. Potter, Dittman, and Bradley believe counts in 100 cells suffice in most cases. Moss, in an effort to determine how many leucocytes must be examined, has decided that after counting the bacteria in three hundred cells there is still an error of ten per cent; and when 50 cells only are counted his experiments show a variation of from .8 per cent to 30 per cent. Most investigators determine the number of bacteria taken up by at least 100 leucocytes.

Large numbers of leucocytes must be examined because of the variability of the number of bacteria taken up by the individual leucocytes. Cells which are apparently normal as far as can be determined by staining and which may contain an average of from 5 to 8 bacteria per leucocyte, will phagocyte from 20 to 30 microörganisms at a maximum while other leucocytes may take up none. This difference in the number of bacteria taken up by individual leucocytes has been supposed to be due to the leucocytes themselves and to clumps in the bacterial emulsion. Walker has suggested that the leucocytes be kept constantly at a temperature of 37° C., and that they be well mixed after incubation with serum and bacterial emulsion. Undoubtedly all investigators have mixed the leucocytes, bacteria and serum well after incubation and before making the spreads to be examined. This procedure, however, does not overcome the differences in numbers of bacteria per leucocyte, as is shown in Walker's tables, in which the variations in numbers of bacteria ingested range from 1 to 15, 2 to 16, 1 to 17, and 4 to 16 in four sets of 25 leucocytes examined. It cannot be concluded from this that there is a uniformity of phagocytosis, although Walker's tables do show a uniformity of phagocytic index in groups as small as 25 leucocytes. It is evident, however, that if 25 leucocytes only were to be examined, the opsonologist might, after he had examined

24 leucocytes, have some choice for the 25th one to he examined, which choice would change the result from 1 to 17. Based on this selection the opsonic index could be materially lowered or raised as might be desired.

In one of the early contributions on the subject of opsonic index, Simon, Lamar, and Bispham recommend that in making the spread of the mixture after incubation, the spreader slide be merely kept in contact with the blood without touching the lower slide, for, "Otherwise it may happen that most of the leucocytes containing organisms, are carried to one end, while only the empty cells are found in the intervening space." In the laboratories of the Johns Hopkins Hospital, Cole, Moss, and Jeans and Sellard have made determinations of phagocytic indices determined in different parts of the slide. It was found that the leucocytes collected near the edge of the smear contain decidedly more bacteria than those toward the center of the slide. By dividing a slide into three zones they found that at the end of the smear the leucocytes contain more bacteria than in the first and middle zones. To explain these differences these men assume that the polymorphonuclear leucocytes containing the largest number of bacteria are so increased in size that they are drawn to the end of the slide while the smaller ones drop out earlier. These results indicate that, contrary to Wright's suggestion, one ought not to determine the number of bacteria at the end of the spread.

Wright's method of the determination of the opsonic index has yielded such marked differences in results that more work on this subject seems justifiable. It must be remembered that the opsonic index is of no value whatever unless carried out with the greatest care and by some one who has had considerable experience. Modifications of Wright's technique have arisen with too much rapidity to make it possible to lay down any definite rule for the determinattion of the opsonic index. However there are only few modifications that have made it possible to get more reliable determinations of Wright's opsonic index.

CHAPTER V.

OPSONIC INDEX IN HEALTH AND DISEASE.

The active participation and function of the white blood cells in the protection of the body against infection was first carefully studied by Metchnikoff. He regarded the leucocytes as cells whose object is to remove from the body and its tissues bacteria and foreign material. On this he based his theory of immunity. It is not strange that Leishman, Wright, and others should have suspected differences in phagocytosis in health and disease. In 1902 Leishman, and especially Wright, emphasized the difference in phagocytic ratio observed in individuals with infections running favorable or unfavorable courses. While trying to find a method by which to control the administration of vaccine, Wright discovered opsonins. With the discovery of opsonin and the development of a method for the determination of the same, it was evident that the opsonic index in health and disease should be investigated. Wright found that in individuals with localized or chronic infections there is usually a decrease in the opsonin content of the blood. This decreased opsonin content as determined by the opsonic index, according to Wright's theories, is due to the fact that in local infections but little of the bacterial substance is absorbed, and so gives rise to but a small amount of active immunity. When this is the case the local infection becomes chronic.

By the method of the determination of the opsonic index, Wright has demonstrated that following the injection of vaccines made from the cultures of infecting organisms, there is first a drop in the opsonic index and later a rise of the same. The drop in the index he calls the "negative" phase and the rise the "positive" phase. The observation of negative and positive phases was made by Ehrlich in 1892, who found that following in-

jection of tetanus toxin there is at first a slight diminution in the anti-toxin in the milk of the animals injected while later there is an increase in the amount of anti-toxin. Likewise Solomonson and Madsen found that similar results are produced in the blood when diphtheria toxin is injected. Agglutinins, precipitins, bacteriolysins, after injection of killed cultures of bacteria, are also at first decreased and later increased.

Wright and Douglas did not, as has been supposed by many, find that all pathogenic bacteria are sensible to opsonins. They divide the pathogenic bacteria into four groups:

a. Those bacteria that are sensible to bactericidal, bacteriolytic and opsonic action of the normal human blood. B. typhosus and Spir. choleræ belong to this group.

b. Those bacteria that are sensible to opsonic and especially bactericidal action of normal blood serum. Examples of this group are to be found in B. coli and B. dysenteriæ.

c. Those bacteria that are definitely sensible to opsonin but not to the bactericidal action of normal blood. B. pestis, Mic. melitensis and Mic. pneumoniæ belong to this group.

d. Those bacteria that are insensible to the opsonic and bactericidal action of normal human blood serum. Examples of this group are found in the bacillus of diphtheria.

Practically all of Wright's observations on opsonic immunization were determined in man. In the human, following the injection of killed cultures, the negative phase is usually of short duration, lasting from 24 to 48 hours. The positive phase may not, but usually follows the negative phase and lasts from 3 to 4 days to two weeks. In active immunization Wright reasons the curve of protected substances may be affected in different ways. If the dose of vaccine injected is very small, the negative phase may be omitted, while if a very large dose is injected it will be marked and the highest index in the positive phase may not be as high as it was at the time of injection. Usually in active immunization a number of injections of vaccine must be made. These may produce one of three effects on the opsonic index:

a. If injections are made after the index has returned to the normal, the index curve may show a series of indices above and below the normal. This is what happens, according to Wright,

when the individual is allowed to recover completely between injections. The curve is illustrated in Fig. 12A.

b. The injections may be given at such times that one negative phase is superimposed upon another negative phase and in this way produce a cummulative effect in the duration and degree of the negative phase. This result is obtained according to Wright when active immunization is pushed rapidly or forced. The curve of protective substance is shown in Fig. 12B.

c. The injection may be made at such times and in such amounts that there is a summation of positive phases. This is the result sought for in immunization. The curve representing the amount of protective substances in this case is represented in Fig. 12C.

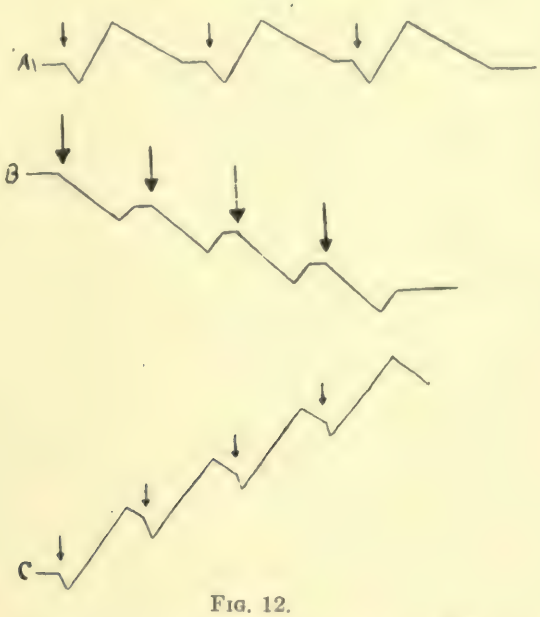

FIG. 12.

Wright, in his treatment of infections by the injection of vaccine, tries to inject such doses at such intervals of time as to give him a continuous "high tide phase." It is to be noted, however, that even with the most favorable summation of positive phases Wright has not obtained an index as high as 4.0 except in rare instances. This, of course, is entirely out of proportion with the amount of other immune bodies produced by active immunization.

Before the principle and methods as formulated by Wright can be accepted it is necessary to decide (a) that opsonins are of

importance in immunity and that the method of determining the opsonic index is accurate; (b) that in the natural recovery from an infection the opsonic index changes, as Wright aims to change it, by the injection of bacterial vaccine; and (c) that the injection of vaccine produces the changes described by Wright.

IMPORTANCE OF OPSONIN IN IMMUNITY AND ACCURACY OF THE METHOD OF THE DETERMINATION OF THE OPSONIC INDEX.

Spontaneous phagocytosis has been described from time to time. With the introduction of Wright's opsonin theory and the development of the technique of opsonic index determination, phagocytosis has been found to occur, in most cases, only when fresh serum is present. Neufeld and many others have found that contrary to Wright's statement phagocytosis is not entirely dependent upon opsonins, for bacteria with little virulence may be taken up by the leucocytes even though no serum is present. The existence of opsonin, however, and its relation to phagocytosis can no longer be doubted; the part it plays in immunity has not yet been satisfactorily demonstrated. Many of the objections made to Metchnikoff's phagocytic theory of immunity present themselves in the opsonic theory of immunity. It has been found that highly virulent bacteria are not nearly as readily ingested by leucocytes as are less virulent ones. Bacteria engulfed by leucocytes are not always dissolved and disposed of for it has been demonstrated by various investigators that certain species of bacteria produce or secret substances that are antogonistic to and actually kill leucocytes. Even if it is accepted that opsonin plays an important part in immunity, the opsonic index determined by Wright's method, need not necessarily be an index of the patient's immunity to certain infectious microörganisms. Agglutinins, bacteriolysins, and anti-toxins, all of which are substances better known than are opsonins, are only circumstantial evidence of greater or less importance in the complex phenomenon of immunity. The body has various means of defense against bacterial invasion and action so that the assumption that all the protective substances and means against bacterial diseases and infections manifest themselves in such a manner that they can be determined from the opsonic index seems unreasonable.

In the previous chapter the accuracy of the method for the determination of the opsonic index has been criticised. It is certain that the method is complicated, requires a considerable amount of experience, and with all the modifications and improvements in the technique is still subject to such great error that even in experienced hands the determinations of the opsonic index must be regarded as of doubtful value.

CHANGES IN THE OPSONIC INDEX IN NATURAL RECOVERY FROM AN INFECTION.

Wright, at various times, has reported observations on the phagocytic reaction and opsonic index in infections running favorable and unfavorable courses. He has found that recovery and increased opsonic index accompany each other. Hektoen has reported that in pneumonia the opsonic index is at first low and rises as the patient's condition improves so that at the crisis the index is above normal. In patients that have a persistently low opsonic index in this disease death usually follows. Tunnicliff has found that in scarlet fever the opsonic index for streptococci is below normal early in the disease and, as the acute symptoms subside, it rises above normal. Later on it again becomes normal. Ruediger has found in erysipelas a sharp rise in the index for streptococci as the temperature begins to fall. Hamilton isolated pseudo-diphtheria bacilli in 75 per cent of cases of acute otitis media and found wide variations in the index for this organism in these cases. Clark has found that the typhoid opsonic index drops before a relapse in typhoid fever.

The writer in a study on the opsonic index in erysipelas made observations on the changes in the opsonic index in unvaccinated patients to determine the relation between the opsonic index for Streptococcus erysipelatos and recovery from, migration, recurrence and desquamation in erysipelas. The most instructive of these cases are the two which follow:

Case I.—S., a man, aged thirty-eight years, who had a migratory, recurrent erysipelas of the face, ears, scalp, and neck, was admitted to the hospital on the fourth day of his disease. When his face was involved, his index was 0.7; when it was desquamating, the index had risen to 1.3; when his face was again involved

seven days later, the index was 1.4; and when it was desquamating again, the index had dropped to 0.8. When his neck became involved, one day after his face began to desquamate for the second time his index was 1.0; two days later, with an index of 1.1, his back became involved, and three days later, with an index of 3.2, his shoulders and neck were again involved, and two days later, with index of 1.1, the back and shoulders began to desquamate. (Chart I.)

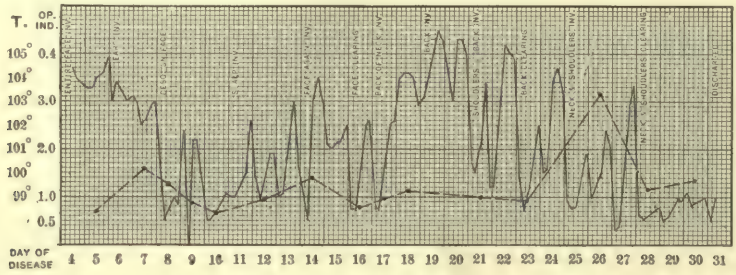

CHART I.—Temperatrue (unbroken line) and opsonic index (broken line) in a case of erysipelas (Case I).

Case II.—A., a man, aged twenty-four years, suffering with erysipelas of the face and ears, was admitted on the eighth day of the disease with an index of 0.9. The next day his temperature dropped and desquamation began; his index was found to be 1.1. Subsequently the index, though the patient was perfectly well, remained below unity. (Chart II.)

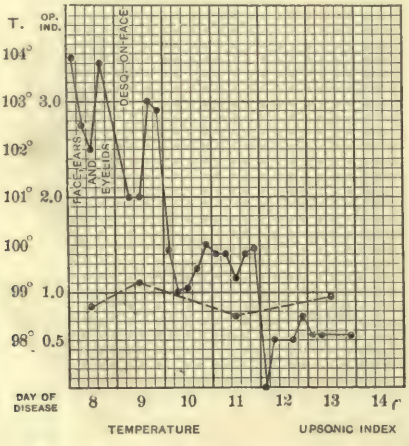

CHART II.—Temperature (unbroken line) and opsonic index (broken line) in a case of erysipelas (Case II).

In these two cases there has been no constant change of index corresponding with desquamation and recovery.

To further study the relation of the opsonic index to the course of the disease, the indices of all patients who had received no vaccine were tabulated with regard to the day of the disease, and an average opsonic index for each day was determined.

TABLE SHOWING THE OPSONIC INDEX AT THE TIME OF ADMISSION, BEFORE KILLED CULTURES WERE INJECTED, AND THE DAY OF THE DISEASE.

Day of Disease.										
1	2	3	4	5	6	7	8	9	10	11
0.6	1.2	0.6	0.7	0.7	0.4	0.9	0.8	1.1	0.6	1.0
0.3	1.1	1.1	1.1	1.0		1.1	0.8	0.9		0.7
	1.0	2.4	1.4	0.9		0.6	1.3	1.7		1.0
		1.7	1.5	0.6		1.6				
			0.7	1.1						
			1.0							
			1.2							
0.45	1.1	1.45	1.1	0.86	0.4	1.05	0.96	1.23	0.6	0.9
Average.										

The composite chart indicates that erysipelas causes an increase of the opsonic index which reaches its maximum about the third day of the disease, and is followed by a gradual fall. The subsequent course of the chart represents, in very large part, observations made upon recurrent, migratory, and complicated cases. Ruediger found that the index was high during an attack of erysipelas.

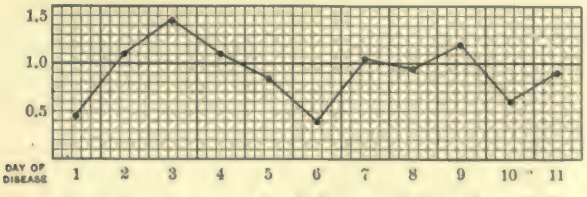

CHART III.—To show the average opsonic index and the day of disease before killed cultures of streptococci were injected.

It is to be noted that the determinations made and represented in the last curve do not represent the index in any one case.

The indices in individual cases are so variable and show such great irregularity that determinations of the opsonic index in any case give little indication of the severity of the disease and is of no value in prognosis.

Most investigators have found that in normal individuals the curve of opsonic index is very irregular. Moss in thirteen observations on one individual determined indices which varied from 0.52 to 1.95 and in another from 0.42 to 1.66. Bolduan reports observations in which one patient's serum varied in opsonin content as determined by Wright's opsonic index from 0.82 to 2.50 in seventeen days. Inasmuch as the opsonic index during infections shows no greater variation than during health it can hardly be accepted that determinations of opsonic indices are of much assistance in diagnosis and prognosis in disease.

Up to the present, most investigators have found that in the natural course of infection there is no regular curve of the opsonic index and that in the natural recovery from an infection the changes in opsonic index are not those which Wright attempts to obtain by injection of killed cultures of bacteria.

CHANGES IN OPSONIC INDEX PRODUCED BY INJECTIONS OF KILLED CULTURES.

Wright has observed that the injection of killed cultures is usually followed by a change in opsonic index for the organisms injected. If the amount of killed cultures injected is large, he states there is a marked negative phase, after which there may be either a marked or slight increase in the opsonic index. If the amount of killed cultures injected is small, the negative phase may be small or absent entirely and following this there may or may not be a positive phase. Wright has attempted to regulate injection of killed cultures so as to get as small a negative phase and as large a positive phase as is possible. To accomplish this, he has determined the number of killed cultures in emulsions and has injected certain numbers of bacteria at a dose, the number varying with the species of the organism and the opsonic index changes obtained by their injection. In some cases Wright has resorted to autoinoculation, which is accomplished by massage or manipulation of the infected part. This partly removes

the toxins from the diseased tissues to healthy tissues where immune substances are actively produced. The amount of massage and manipulation Wright regulates by the changes produced in the opsonic index.

While numerous investigators have reported results of vaccination based on opsonic index determinations and have obtained, after injections, almost always a uniform rise in the opsonic index, most investigators have not found this to be the case. Even though Wright claims to have obtained changes in opsonic index showing considerable regularity, yet on examination of his charts one sees curves of most marked irregularity; the index is not constant in normal individuals; shows no consistent increase or decrease in different stages of infection, and is influenced by many non-specific processes as menstruation, exercise, food, etc. Numerous investigators have found that even though there may be first an increase in opsonic index following vaccination, yet this is soon followed by a fall of the index and still improvement of the condition will be observed.

The results obtained on determinations of opsonic index in health and disease are so variable that its importance as a means of diagnosis, prognosis and indication of actual immunity must be doubted. It is definitely certain that the index is of no value whatever unless carried out carefully by one having had considerable experience in its determination.

CHAPTER VI.

THE NATURE OF OPSONINS

The theories of Wright concerning opsonins have received attention chiefly because of the apparent value of their quantitative determination in health and disease and in the regulation of dosage and interspacing of injections of bacterial vaccines. Numerous investigators, however, have attempted to determine their importance in immunity, their structure, and the conditions which influence their action.

Wright has found opsonins, the substances which prepare bacteria for phagocytosis, present in the serum of normal, diseased and vaccinated individuals. Neufeld and Rimpau, on the other hand discovered substances which prepare bacteria for ingestion by leucocytes, present in certain **immune** serum. Inasmuch as both the **opsonin** of Wright and the **bacteriotropin** of Neufeld and Rimpau perpare bacteria for phagocytosis, they are by many considered as identical substances. Various investigators, while admitting that these substances may be identical in some sera, do not regard them as the same substances in all sera. Wright has regarded opsonin as an important factor in immunity, but has made no distinction between the opsonin present in normal and immune sera. Numerous investigators, however, have demonstrated and emphasized certain differences in the bacteriotropic substances in normal and immune sera.

SPECIFICITY.

While Wright and Douglas did not discuss the question of specificity of opsonins, still it is evident that they consider opsonins as specific. The opsonic index in disease, according to Wright, shows certain deviations from the normal for certain microörganisms, while for other organisms it deviates but little from the opsonic index obtained with normal serum. On this observation

Wright has based a method for the diagnosis of certain microorganisms in infections. This can, however, only be regarded as logical if opsonins are specific. Bulloch and Western found that in normal serum, by absorption tests, the specificity of opsonin for B. pyocyaneus, B. tuberculosis and staphylococci could be determined. Muir and Martin, Simon, Russell, Potter, Dittman and Bradley, and others, have found that **normal opsonins** are not specific. Muir and Martin, and Russell, however, by absorption tests have determined that **immune opsonins** are quite specific. Simon, however, has not been able by investigation to prove the existence of specific immune opsonin.

EFFECT OF HEAT ON OPSONIN.

Wright has consistently assumed that normal and immune opsonins are identical and has preferred to regard all opsonins as thermolabile. There are, however, on the other hand, a large group of observations which show that while opsonins in immune serum resist a temperature of 55° C. for one hour, opsonins in normal serum will no longer be able to prepare bacteria for phagocytosis by the leucocytes after such an exposure to heat. These observations show plainly that immune opsonin is thermostable, while normal opsonin is thermolabile. The basis for Wright's assumption that they are all thermolabile is not evident, inasmuch as Wright and Reid have shown that in the serum of certain patients suffering from tuberculosis there is a thermostable opsonin. On this observation Wright has based a method for the diagnosis of tuberculosis, which can only have for a foundation the assumption that in tuberculosis specific immune bodies which are heat resisting, are produced. From the various investigations reported, normal and immune opsonins manifest a marked difference in ability to withstand heat.

STRUCTURE OF OPSONINS.

Opsonins have, by numerous investigators, Savtchenko, Besredka, Loehlein and Dean, been regarded as identical with amboceptors (fixateur). Muir and Martin have shown that not every immune body produces an opsonizing effect. Hektoen has, from a series of experiments, decided that opsonins are distinct substances or anti-bodies. Neufeld and Rimpau, Neufeld and

Toepfer, Keith, Bulloch and Atkin, agree with Hektoen and Wright and Douglas that opsonic action is due to the presence of hitherto unknown distinct bodies.

Before this can be accepted, however, it will be necessary to repeat many of the experiments that have been made, inasmuch as in England and America especially, these investigations were made at a time when no distinction was made between normal and immune opsonins.

The existence of normal and immune opsonins is now quite generally accepted. Neufeld in a consideration of the causes of phagocytosis, states that he believes bacteria and foreign bodies are only taken up by the leucocytes when the latter are stimulated. He bases this assumption on the phagocytosis of red blood cells by leucocytes, which occurs only when a special haemotropic serum is present. In the haemotropic serum, according to Neufeld, the physico-chemical condition is so changed that a part of the body is modified so as to serve as a stimulus or appetizer for the phagocytes. Virulent organisms dissolve with greater difficulty and give off less appetizer, and because of this there is less phagocytosis of virulent organisms than of organisms with decreased virulence. Spontaneous phagocytosis, according to Neufeld, is due to changes in the cell, one of these changes accidentally stimulating the leucocytes to phagocytosis. In immunization Neufeld believes a specific immune substance is produced, which substance modifies bacterial or other cells so that they will serve to stimulate the leucocytes to phagocytosis. Immune opsonin or bacteriotropin, as he prefers to call it, is a thermostable substances which does not require complement. Normal opsonin, on the other hand, is believed by Neufeld to produce its action because of normal amboceptor and complement, which gently dissolves bacteria and in this way stimulates the leucocytes to phagocytosis. The assumptions of Neufeld are borne out by numerous investigators.

Immune opsonins resist temperatures up to 55° for one hour, 65° C. at times not being sufficient to destroy their action. If the opsonizing action of immune serum is once lost it cannot be regained by the addition of fresh complement. Muir and Martin have found that inactivated immune opsonin absorbs little or no complement. Because of the properties of immune opsonins, they

are generally regarded as belonging to the anti-bodies of the second order of Ehrlich. They apparently possess two groups, the opsonophore and the haptophore. Of these the opsonophore group is destroyed by heat, age, acids, etc. It is thus seen that immune opsonins resemble the agglutinins and precipitins in structure and by some investigators have been thought to be identical with agglutinins.

Normal opsonins, though not acknowledged by Wright to be different from immune opsonins, have characteristics by which they differ from immune opsonins. Normal opsonins resemble complement in that they are absorbed or fixed by sensitized and non-sensitized bacteria, blood corpuscles, specific precipitates and indifferent bodies, and exhibit thermolability and susceptibility to deterioration by age. Noguchi has found that normal opsonins resemble complement in that they are highly labile bodies, loose their action on standing several days, are preserved for a long time when present in blood in a dry state, and in this condition can be heated to 135° C. without destruction of their functions. Recently Muir and Martin, Levaditi and Inman, and Hühne and Neufeld have ascribed the action of normal opsonins to complement. Cowie and Chapin have found that normal guinea pig serum restores the opsonic power to normal serum which has been heated to 55° C. They believe from their experiments that opsonins (normal?) exert their action because of an amboceptor-complement group. Hektoen has recently published results of experiments from which he concludes that the activating element is free from the opsonin and therefore he believes that opsonins belong to the third order of anti-bodies of Ehrlich.

It now seems quite definitely established that the action of normal and immune opsonins is due to entirely different factors in immunity, and that immune opsonins are distinct anti-bodies probably belonging to those of the second order of Ehrlich.

RELATIONS OF OPSONINS TO LEUCOCYTOSIS.

No consistent relation has been found to exist between changes in opsonic index and general or localized increase in the number of leucocytes. Simonds and Baldauf have recently found that following an injection of heated bacteria there is a decrease in leucocytes after which there is a marked increase in leucocytes with an

ultimate return to the normal. These changes in the number of leococytes they find precede the changes in the opsonic index. When the index is at its height the number of leucocytes has returned to the normal. Various methods designed to increase the number of leucocytes have been resorted to but have all been of little avail in increasing the opsonin content in the blood as determined by Wright's opsonic index. If, however, the leucocytes can be stimulated to phagocytosis an increase in the number of leucocytes in the infected region must certainly be of value. Opsonins are by some supposed to originate in the leucocytes. The importance and possibility of this becomes more evident from the work of Peterson and Hiss and Zinsser. Potter believes that the opsonic index varies with the source of leucocytes.

RELATION OF OPSONINS TO STIMULINS.

Metchnikoff has described a series of experiments in which the introduction of serum, either from normal or immunized animals, greatly increased phagocytosis. This action, he supposes, is exerted on the leucocytes and is of great importance in phagocytic immunity. The substance in the serum which he regards as stimulating leucocytes he calls "stimulin." Stimulins are probably the same as opsonins, even though up to the present time, in the observations reported, their action is on the leucocytes and not on the bacteria.

OPSONINS AND AGGRESSINS.

Endotoxins or the aggressins of Bail, have by some been supposed to account for decreased phagocytosis or low opsonic indices obtained with some sera. Aggressins are supposed to injure the leucocytes and inhibit their action. The part the endotoxins play in preventing phagocytosis has not yet been determined. Dorr, Sauerbek and others have shown that aggressins are not difinitely specific. Neufeld from experiments has decided that the lack of phagocytosis with virulent organisms does not depend upon the injury to leucocytes and for this reason does not believe that decreased phagocytosis is due to the action of aggressins.

INFLUENCE OF CHEMICALS AND REACTION ON OPSONINS.

Various salts, even in small amounts, influence opsonic action markedly. Noguchi has shown that in normal serum, reaction exerts considerable influence on phagocytosis, it being most marked when the serum to be tested is neutral in reaction.

NON-BACTERIAL OPSONINS.

Opsonins have been found to be produced for other cells than bacteria. Hektoen has reported opsonins for blastomycetes and red blood cells. Likewise it is possible to get marked phagocytosis of inert particles of charcoal, stains, etc., upon the addition of certain sera.

CHAPTER VII.

VACCINE THERAPY

For centuries it has been known that following an attack of certain acute infectious diseases there remains a certain loss of susceptibility to the contraction of a second attack of the same disease. Early in the eighteenth century this experience was utilized by vaccination for small-pox. When it was found, about the middle of the nineteenth century, that many acute and infectious diseases have for their etiological factor certain micro-organisms known as bacteria, attempts were made to induce immunity by inoculation of killed or attenuated cultures of these organisms. Pasteur in this way successfully immunized against chicken cholera, but while a certain amount of success by the injection of killed cultures was obtained, still it was found impracticable for reasons that are not understood nor can be considered here. Recently, however, Haffkine has successfully immunized or vaccinated against cholera and Pfeiffer and Wright against typhoid fever. It would prove impracticable to employ active immunization against all organisms with which man may be infected, for this would require frequent injections of all kinds of organisms. Active immunization to be applicable generally must be such that it can be instituted after infection has occured. In this Pasteur was successful in the disease of hydrophobia. It was found, however, that when the method of active immunization is begun after infection has occured, there is a superimposition of a mild form of the disease upon a severer form, thus accentuating the disease. This observation lead to a relatively long period of cessation of this method of treatment.

In recent times active immunization after contraction of the disease, has again become important. This has been due espec-

ially to the efforts of Wright. It is important to note that the method of vaccination was used by Wright before he had discovered opsonins. From his later observations Wright has decided that opsonins are of the utmost importance in immunity to some organisms, and are decreased in amount during most infections but that they can be increased by the injection of bacterial vaccine.

BACTERIAL VACCINES.

It was stated in a former chapter that in bacterial infections the infected body absorbs bacterial substances and products. According to the amount of substance and products absorbed immune substances are produced. When much is absorbed sufficient immune substance is usually produced to cure the disease, but when little is absorbed not enough immune body is produced to cure the infection which then becomes chronic. To produce enough immune substances to affect a cure from an infection Wright and Douglas have employed the well known principle of active immunization. The method they have suggested is to actively immunize by injection at the proper time, of proper doses of killed cultures of the organisms causing the infection. The effects of injection of killed cultures or vaccines according to these investigators is to produce certain changes in the opsonin content. Wright and Douglas attempt to immunize by giving injections of such numbers of bacteria as will produce only a slight negative phase which lasts only a short time and is followed by a rise in opsonic index which lasts for some days. When the index begins to fall after the subsidence of the positive phase, another injection is made and the effect on the opsonic index is determined. An attempt is made to inject such doses at the second and subsequent injection that there shall again be only a slight negative phase and that during the positive phase the opsonic index shall be higher than following the first injection. An effort is made to keep the opsonic index of the individual above normal. If the index shows a marked drop after any injection, the dose has been too large. By means of vaccines Wright claims that the antibacterial power of the blood can be increased for any microbe invading the body. In 1906 Wright was able to say that he had by this method achieved **uniform** success.

DOSAGE.

Wright has, by means of the opsonic index determined the proper dosage for the different bacterial vaccines and while he as not stated that definite amounts must be used, still he has suggested certain doses. His principle in dosage is to use the smallest dose that will give a good rise in the opsonic index and never to increase the amount injected until it has been ascertained that the doses which have been injected have been too small to produce a good rise in the opsonic index.

The doses of vaccine recommended by Wright are as follows:

Bacillus coli	5 to 50 million bacteria
Micrococcus gonorrhoeæ	5 to 50 million bacteria
Micrococcus pneumoniæ	10 to 50 million bacteria
Bacillus pyocyaneus	5 to 5 thousand million bacteria
Micrococcus pyogenes	50 to 1 thousand million bacteria
Streptococcus pyogenes	10 to 25 million bacteria
Bacillus tyhosus	5 to 50 million bacteria
Koch's New Tuberculin	1-1,000 to 1-400 milligrams

These doses are by some considered purely arbitrary, because the personal characteristics of the individual to be immunized, the duration, extent and severity of the infection, must all be considered in active immunization. While these doses may be arbitrary they at least offer some guide as to the number of organisms to be injected at a time. At times in active immunization the injection of vaccine is accompanied by injection of specific immune serum. This will pave the way for larger doses and more powerful vaccine injections.

Injection of the doses recommended by Wright usually produces no constitutional symptoms. Wright has stated that at times malaise, slight general muscular pain and headache are observed on the day following the injection but as the positive phase comes on there is a marked buoyancy of spirits and stimulation. Most investigators on this subject have never observed untoward symptoms when the small doses recommended by Wright are injected, while others have at times observed a rise of temperature, nausea, vomiting, etc. According to the principle of active immunization probably the greatest amount of immunity results when there is some reaction for in active immunization the individual or animal undergoes a modified or less severe form of the disease.

The point of injection of bacterial vaccines is considered by Wright to be of importance in the success of vaccination. Those regions of the body are selected where there is rapid absorption by the lymph and blood. It has been found that on subcutaneous injection greater amounts of immune body are formed than upon intravenous injection. This is especially illustrated in the production of certain haemolytic sera.

If Wright's method of vaccine treatment of bacterial diseases is to be judged it must be remembered that the injection of killed cultures did not originate with Wright, but that Wright has added opsonic index determinations to control the time and doses of these injections. Opsonic index determinations have, however, been found to be open to so many sources of error, of such great variability in health, disease and after injections of vaccines, that many clinicians have decided upon the dosage and inter-spacing of injections by clinical symptoms rather than based upon opsonic index determinations. Most clinicians have, in the first injection, used the dosage recommended by Wright but in subsequent injections have used such amounts as seemed warranted and indicated. Hektoen, adhering to the reliability of opsonic index determinations, holds that the index is of diagnostic and prognostic importance, while Park, Cole, Bolduan and others regard the index as unreliable for such purposes.

ORGANISMS USED IN VACCINATION.

Many of the species of pathogenic bacteria have been used in active immunization. Two kinds of vaccine treatment have been employed, one consists of the injection of killed cultures of the causal organisms and the other of the autoinoculation from the infected focus by means of massage and manipulation.

In the injection of vaccines, so called "stock culture" vaccines and "personal" or "autogeneous" vaccines have been used. It has been found that for some organisms, vaccines made from stock cultures will give as good results as will personal or autogeneous vaccines. Stock vaccines have the advantage that no time need be lost in the preparation of the vaccine, and for nearly all of the species of bacteria causing infections amenable to treatment by active immunization, stock vaccines are equally as good as autogeneous vaccines. The principle exceptions are the vac-

cines for Streptococcus pyogenes, Mic. pneumoniæ and, according to some, for the Mic. gonorrhoeae.

Some investigators have used vaccines containing several species of bacteria. Serum and vaccine manufacturers have prepared vaccines suitable for active immunization against one or many different sepcies. Wright at one time made no particular effort to identify the species of the causal organism, simply making a vaccine from the cultures obtained by inoculation with material from the lesion. This is open to many objections, for at times the causal organisms may be such that it will not grow on ordinary media or else the causal organisms may not grow as well as secondary invaders and saprophytic species. Because of the mixed and undetermined vaccines used in immunization it may frequently happen that those organisms, for which immunization is necessary or beneficial, are not injected.

In all cases it ought clearly to be remembered that if benefits are to be derived from active immunization the vaccine must consist of a suspension of the causal organism. To determine these, microscopic examinations of the material from the lesion, and isolation of the organisms when possible ought to be made. This cannot be too strongly emphasized, especially to the practitioner who can buy from the druggist vaccines said to be good for boils, acne or whatever the condition may be, while as a matter of fact the vaccine may not contain the organism causing the particular lesion which is to be recovered from as a result of active immunization. This has undoubtedly been the cause of some of the failures observed by some clinicians and has, as was the case with tuberculin, decided the practitioner against the use of bacterial vaccines in certain cases of infection in which vaccine might have been of value.

PREPARATION OF BACTERIAL VACCINES.

While killed cultures of bacteria have for some time been used in active immunization, the amounts injected had been only indefinitely determined. Wright and Douglas originated and described a method for the preparation and standardization of bacterial vaccines to be used in immunization. The causal organisms according to this method are grown on artifical culture media after which they are suspended in sterile salt solution. The num-

ber of bacteria per cubic centimeter of the suspension is determined by drawing into a capillary pipette one volume of the bacterial suspension, and one or more volumes of fresh blood from a puncture in the finger. These volumes are then mixed well and a drop of the mixture placed on a clean slide. With a spreader which is placed in front of the drop, a smear is made. This is then allowed to dry, is fixed and stained with a suitable stain, usually Loeffler's methylene blue. The slide is then counted by determining the number of red blood cells and bacteria in from ten to twenty-five fields of the high power of the microscope. Normal human blood contains five million red blood cells per cubic millimeter. After having established the ratio of red blood cells to the bacteria in the suspension, it is easy to determine the number of bacteria per cubic millimeter or cubic centimeter. The tube containing the bacterial suspension is now sealed and heated in a water bath, to 60 or 65° C. for one hour. After killing the bacteria in the suspension, a dilution, suitable for injection, is made in a bottle containing 50 c. c. of sterile salt solution. The bottle is closed with a rubber cap and sufficient carbolic acid or lysol is added to make an ultimate dilution of 0.5 per cent. After this the vaccine is tested for sterility on suitable culture media. To obtain vaccine from the bottle a drop of pure lysol is placed on the rubber cap. This is done to sterilize the outer surface of the rubber cap. Then the sterile hypodermic needle is inserted through the cap, the bottle turned up and the piston of the syringe is withdrawn until the desired amount of vaccine has been taken into the barrel of the syringe.

CONTROL OF INJECTION.

While Wright and his pupils have claimed to regulate the dosage and inter-spacing of injections entirely by the opsonic index, others have found the opsonic index too unreliable to serve as a guide in the administration of bacterial vaccines. Wright and his pupils have not always based their decisions on dosage and time for injection, on determinations of the opsonic index. It frequently happens that at the time of the first injection of vaccine their patients have an opsonic index well above normal. In most cases the treatment is based on determinations made by serum drawn twenty-four hours earlier. The time for the second

and subsequent injection is not always controlled, even by Wright and his pupils, by the opsonic index.

In accepting opsonins as important factors in immunity other immune substances must not be lost sight of. Various investigators have designed methods by which the action of agglutinins and bacteriolysins is to be inhibited, so that opsonic indices may be determined and used as a guide for the dosage and inter-spacing of injections. The justifications for such regulation of dosage and determination of the proper time for injection is not evident. In a patient suffering with cerebro-spinal meningitis, from the spinal fluid of which Mic. meningitidis was cultivated, the writer tried to govern the dosage and inter-spacing of injections of meningococcus vaccine by the opsonic index. After three to four minutes of incubation of the mixture of leucocytes, meningococcus suspension and patient's serum, all of the organisms were found to be dissolved. It is evident that lysins in this case were probably of more importance in immunity than were opsonins. Clark has proposed that in determining the opsonic index of serum of typhoid patients, the serum be heated to 56° C. in order to destroy the lysins for the typhoid bacillus. By following out this method Clark finds that relapses follow upon a drop in the opsonic index. How much more important would it probably be to determine the curve for lysins in a case where lysins are present in such amount that it is necessary to destroy their action before opsonic index determinations can be made? This would seem more rational, especially because our knowledge concerning lysins in immunity is greater than it is concerning opsonins, which may or may not play any part in immunity.

According to the determinations of the opsonin content of the blood, Wright and his pupils have found that the opsonic index begins to rise two to five days after injection, and usually reaches its height after about five to eight days. In most cases adherents of the opsonin theory make injections every four to eight days. The appearance in the blood, however, of the better known anti-bodies comes somewhat later. Bacteriolysins are found to be present at times in the spleen twenty-four hours after injection of culture. They do not, however, appear in quantities in the blood until five to nine or fourteen days later. Agglutinins are found to be present in the blood from eight to twelve days

after injection of cultures. Typhoid agglutinins usually do not appear in a typhoid patient's blood until after ten days of the disease have elapsed. Furthermore, it is noted that anti-toxins, bactericidins and agglutinins can be enormously increased by correct inter-spacing of injections and proper dosage. Wright himself has called attention to the value of determining these different substances in immunity before injections are repeated. If injections are made entirely according to the opsonic index, they may be made before the bactericidal and other protective substances have increased in amount, several negative phases may thus be superimposed on each other, and the protective substances be much decreased. With the role of opsonins in immunity in doubt, the difference in sensibility of different species of microörganisms to opsonins established, and the presence of marked bacteriolytic action of certian sera for certain species of infecting organisms observed, the control of the process of active immunization by the opsonic index alone does not seem justifiable. The immune bodies of importance in immunity to the particular species of bacteria ought to be established so that the dosage and interspacing of injections could be regulated by determinations of the presence of these substances. Meakins has, on this basis, studied immunity to dysentery bacilli, Streptococcus pyogenes, Micrococcus pyogenes and Bacillus tuberculosis. Such methods would undoubtedly result in a better accomplishment of a higher grade of active immunity.

It is necessary, however, to refer to the great success obtained by Wright, and others following Wright's teachings, in the treatment of bacterial infections and diseases according to the determinations of the opsonic index. Wright has stated that he has not achieved success only in a certain percentage of cases, but that he has had **uniform** success by following his method of active immunization. Ross has given a list of diseases successfully treated based on the determinations of the opsonic index. This list includes furunculosis, pustular acne, sycosis, cystitis, otitis media, empyema, gleet, gonorrhœal rheumatism, acute gonorrhœa, localized tuberculosis, lupus, tuberculosis of the joints, bladder, kidney, epididymis, glands, peritoneum, iris and lungs. Surely a method that will give uniform success for as large a list of dis-

eases as this, is one that must be of the utmost importance in clinical medicine.

Because the methods for the determination of immune bodies are still so inaccurate and in many cases unreliable, and because the defensive agencies of the animal body against bacterial invasion and multiplication are so manifold and complex, numerous investigators have relied upon the clinical control of dosage and inter-spacing of injections of bacterial vaccine. At all events, the value of active immunization by injection of bacterial vaccines will ultimately rest upon clinical results. While it is relatively easy in most cases of infection to tell when complete recovery has taken place, still it is difficult to tell what clinical symptoms and signs are associated with the best method of immunization. The conditions ultimately desired are definitely known, but the conditions in the diseased body which will produce these ultimately desired effects, are little understood, Probably the best illustration of this is seen in the use of tuberculin in the treatment of tuberculosis. Tuberculin has been known and used in treatment for nearly twenty years, and still today it is an open question whether the dosage shall be regulated to give an anti-toxic or an anti-bacterial immunity. Clinical evidence and experience is deceiving as is well demonstrated in the literature on tuberculin.

With some organisms, and in certain cases treated by the vaccine method, excellent results have been obtained when the dosage and interval between injections has been determined by clinical signs and symptoms. The number of cases in which the treatment is controlled clinically is probably larger than the number of those treated according to Wright's method. Many investigators, although determining the opsonic index during the process of immunization, have found that it is quite as safe to administer vaccine when controlled by clinical results as by opsonic index determinations. In clinical control of vaccine treatment, it is aimed to have the dose so small and the injections so far apart that immunization is accomplished without the production of clinical symptoms. The doses that are usually given are those recommended by Wright, though some have used larger doses without the production of any reaction.

In most clinics the doses injected are gradually increased. This is also the procedure recommended by Wright. If this

method is followed, it frequently happens that there may be an exacerbation in the pathological condition. This is especially observed in furunculosis produced by the staphylococci. Frequently following the second injection of a vaccine of this organism a new furuncle will appear. The writer has obtained better results by first injecting the largest dose and then at subsequent injections to give constantly decreasing doses. The reasons for following this method are based entirely upon clinical experience and may be due to the fact that if the second and subsequent injections are larger than the preceding one, too much of the immune substance is used up in combining with the bacterial vaccine injected or there may be an actual hypersusceptibility to these organisms.

The interval between injections when the opsonic index is not determined, lies usually between seven and ten days, which is about the time required for the production of known immune substances. Whenever a reaction follows an injection the interval between injections is lengthened so as to allow sufficient time for the disappearance of all symptoms.

Staphylococcus Infections. The various infections caused by the Micrococcus pyogenes group have been considered most satisfactory for vaccine treatment. In the list of staphylococcus infections treated by bacterial vaccines, Ross includes boils, carbuncles, acne, sycosis, felons, styes, and septic wounds. The results obtained in the treatment of furuncles and carbuncles by killed cultures of the infecting organisms have varied. Probably the best results have been obtained in localized surface infections. In the treatment of these cases it has been found that autogenous vaccines are not necessary. It is, however, important in all cases to determine the species and variety of organisms causing the infection and to use this variety in the treatment of the pathological conditions. The staphylococcus vaccines with which the writer has obtained the best results are those made from different isolation of the same variety of organisms. Thus a Mic. pyogenes aureus vaccine which has given good results is one made from

three isolations, the organisms being isolated from the lesions of chronic marked furunculosis, from an acute boil on the face, and from a patient having multiple pustules on the back of the neck. Most patients suffering with furnucles and carbuncles show a marked improvement within twenty-four hours after injection and some of these patients remain well after that. Everyone who has treated a number of patients suffering with furuncles or carbuncles must have observed that there occasionally is recurrence of these conditions after apparent recovery following upon the injection of suitable vaccines. If Wright's method of vaccination according to the opsonic index is followed out, there are apparently more recurrences than when the injections are given at intervals not less than seven days nor greater than ten days. In all cases the best results will be obtained if a small incision is made in the carbuncle or furuncle twenty-four hours after vaccination. By this method the collection of leucocytes or pus is drawn off, the pressure is relieved and the bacteria ingested by the leucocytes are removed. The advantage of this becomes evident when it is realized that staphylococci secrete a leucocidin, which destroys leucocytes. If the leucocytes containing bacteria can be removed before they are killed the value of phagocytosis in immunity is evident. After this the pus is evacuated daily from the furuncle or carbuncle and the wound is not allowed to heal until it has been found that for several days no more pus is being formed. In addition to draining the furuncle or carbuncle through a slight incision, thorough cleansing of the skin with benzine is of great advantage in preventing the formation of new foci. The doses used in most clinics for these conditions are gradually increased, the first injections usually consisting of three hundred million cocci. As was mentioned before, apparently better results are obtained when at the first injection six hundred million cocci constitute the dose, while the later doses contain fewer cocci, at times only one hundred million. While this method of vaccination does not depend for its results only on the effects of vaccines, still it is a method which gives good results without the formation of large scars.

Acne vulgaris has been successfully treated by vaccination only in certain cases. The value of bacterial injections in this condition was so emphasized by Wright that for a time it was

the common belief among clinicians that vaccinations with killed cultures of Micrococcus pyogenes albus were applicable in all cases of acne. This, however, is not the case. In the lesions of many patients suffering with acne vulgaris, the white skin coccus cannot be found. In such cases either the species of organisms causing the pustules must be injected or else vaccine treatment should not be applied. A number of the cases of acne which have been treated by the writer have failed to show microörganisms that could be cultivated. When the vaccine treatment is used other means of treatment must not be discontinued. Incisions and evacuation of the pustules, cleansing of the skin with benzine, expression of comedones or black heads, the bathing of the infected part in hot water, application of sulphur or other suitable lotions and the regulation of the diet are all of assistance to overcome this disease. Even under these measures the treatment is not always successful.

The treatment of sycosis barbæ or barber's itch with staphylococcus vaccines has not always been as successful as has been reported. Two patients with this disease in the City Hospital in New York City, were treated by Ross with injections of staphylococcus vaccine. No satisfactory results, however, were obtained. The unsatisfactory results obtained by the injection of staphylococcus vaccine is easily explained when it is realized that staphylococci cause secondary infections in only some of the cases of sycosis barbæ. In these cases only can staphylococcus vaccine be of benefit.

Streptococcus Infections. Various kinds of streptococcus infections have been treated by vaccines. Usually these infections are acute and severe and it often is a question whether sufficient time for the production of immune bodies as a result of vaccination will elapse before death or spontaneous recovery terminates the disease. Arthrites, pneumonia, pericarditis, erysipelas, local infections and endocarditis, etc., have been treated by the use of vaccine. At times striking results have been obtained, while in other cases not treated by vaccination there has been equally striking recovery. One needs only to remember the cases of streptococcus infections of the uterus, of septicæmia and erysipelas that recover in a few days without any specific treatment, to realize that it is easy to attribute beneficial results

to the use of streptococcus vaccines in cases in which the same results might have been obtained without them. In 1907, the author treated 37 patients suffering with erysipelas by injections of killed cultures of streptococci isolated from the lesions of erysipelas. From the results obtained it was impossible to determine the value of the injection of the killed cultures of streptococci. While the injection of from twenty-five million to one hundred million streptococci did not prevent migration and recurrence, the apparent shortening of the duration of the disease suggests that streptococcus vaccines are of some value. The effect upon the duration of the disease was found to be uncertain because, during the time of the investigation, no suitable cases remained untreated with which comparison could be made. It is known that the duration of the disease varies in different years. Ross and Johnson have recently reported on the treatment of erysipelas with a vaccine made from streptococcus erysipelatos, and conclude that such vaccine exercises a specific and controlling influence on the course of the disease. These investigators make initial injections of from ten to twenty million killed streptococci, and repeat these injections every day or two according to the clinical results obtained. Criticism of the results obtained is not possible because of the meagre reports of the cases treated by these investigators.

Wright and others have reported successful treatment of streptococcus septicæmia by the injection of streptococcus vaccine. It must, however, be remembered that streptococcus septicæmia is at times recovered from even when no specific treatment is given.

In streptococcus immunization autogenous vaccines have usually been preferred, though Gabritschewsky and others have gotten good results with stock vaccines.

Gonococcus Infections. Probably the best results of the use of gonococcus vaccines have been obtained in cases of gonorrhœal arthritis. Cole and Meakins obtained encouraging results in about twenty cases of acute and chronic gonorrhœal arthritides when treated by injections of stock vaccines of Mic. gonorrhœae. These investigators treated their cases according to the opsonin content of the blood as determined by the opsonic index, but feel that probably as good results might have been obtained had the injection been given every ten days, which was about the

time determined upon by the opsonic index. The doses injected in these cases varied from five hundred million to one thousand million cocci.

Treatment of acute and chronic urethritis by vaccines has given some, but relatively few, beneficial results, and is by no means to be regarded as a sufficient measure to obtain a cure from this condition.

Micrococci pneumoniae Infections. Apparently some good results have been obtained in the treatment of chronic infections with this organism. The dose injected varies from ten million to one hundred million. In acute pneumonia the course of the disease is usually too short to derive beneficial results by means of active immunization.

Tuberculosis. The bacillus of tuberculosis may produce its lesions in almost any part of the body. Bacteriological examination of the lesions produced will vary. In some lesions, especially those localized in the interior of the body such as the glands, joints and bones, the tubercle bacillus alone is responsible for the condition found. In other lesions, as in pulmonary and intestinal tuberculosis, there are present in addition to tubercle bacilli other bacterial species. Active immunization, by the injection of killed tubercle bacilli theroetically will be of benefit in lesions due to the tubercle bacillus alone, while in lesions produced by tubercle bacilli and other species it will not be very efficacious.

In the specifie treatment of tuberculous conditions, various modifications of so-called tuberculin have been used. **Old tuberculin** is the concentrated germ-free glycerine bouillon on which tubercle bacilli have been grown for several weeks. Before filtration the tubercle bacilli are killed by heat. **Deny's bouillon filtrate, "B. F."** differ from old tuberculin in that filtration is done before killing the bacteria by heat. **Tuberculin R, "T. R."** contains the powdered tubercle bacilli suspended in salt solution. Usually 1 c. c. contains 2 m. g. of powdered bacilli. **Tuberculin O, "T. O."** is the supernatent opalescent liquid obtained after emulsifications of ground up tubercle bacilli in distilled water, are centrifuged. **Bacillen Emulsion, "B. E."** is a suspension of ground up tubercle bacilli in fifty per cent glycerine. It contains 5 m. g. in 1 c. c. of suspension.

Our knowledge concerning the effect of tuberculin on the bacillus of tuberculosis and the tissues of the body in which the bacillus is found, is still indefinite. Before tuberculin can be scientifically applied as a therapeutic measure, it is necessary to determine the part played by the moderate reaction in the tissues and what is to be accomplished by immunization with tuberculin.

The reaction in diseased tissues following the injection of tuberculin may be either mild, in which case, there is redness due to hyperæmia and congestion, or it may be marked, producing necrosis and sloughing of the diseased tissues. In all attempts at immunization with tuberculin the mild reaction is preferred because it does not lead to the liberation of tubercle bacilli as a result of the necrosis of tissues, but does furnish to the lesions an increased blood supply.

The conception of the immunization accomplished by tuberculin injections differs, there being two important theories. Koch, Wright and others believe that anti-body, specific to the action of the tubercle bacillus, is produced. This, according to their views, is accomplished through stimulation of all the defenses of the body. In conformity to their views on the objects of tuberculin immunization, tubercle bacilli are injected in the form of "T. R." or "B. E."

Maragliano, Sahli, Denys, Trudeau, and others adhere to the toxin immunization theory, according to which tuberculin injections lead only to tolerance or immunization to the toxin liberated by the tubercle bacillus. In accordance to this theory, old tuberculin as well as other tuberculins are injected, the doses being gradually increased to the highest point of tolerance. The lesion according to this theory is only secondarily effected, there being no acquisition of immunity to the tubercle bacillus.

Tuberculin treatment is usually only undertaken in tuberculosis of the glands, joints and bones, though in some clinics it has also been used in pulmonary and other forms of tuberculosis. Koch would limit its application to such cases as are still curable—that is, have not advanced too far nor are complicated by other bacteria. In deciding upon cases amenable to tuberculin treatment Koch relies upon the body temperature as the indicator. In uncomplicated and curable cases the temperature does not rise much above normal. Various measures have been taken as

guides in tuberculin treatment of tuberculosis. Wright injects only amounts large enough to produce a positive phase in the opsonic index and in some cases of tuberculous glands and joints has accomplished good results by autoinoculation. Trudeau, Denys and others have regulated the dosage and inter-spacing of injections by clinical signs and symptoms. They usually inject 1-1000 m. g. of old or 1-10,000 m. g. of "Bacillen Emulsion." After this, by a long continued treatment they gradually increase the dosage. The doses injected are not increased nor the interval between injections shortened if there are any general or local reactions, as evidenced by fever, local redness and edema. In this method no stress is laid on the absolute amount of tuberculin injected. When the patient has no tolerance to tuberculin, Trudean has usually observed chronic toxæmia and progression of the disease.

Tuberculin treatment has proven especially beneficial in lesions in the joints, bones, glands, skin and urinary bladder, although good results have been obtained in pulmonary and other forms of tuberculosis.

Bacillus coli Infections. Different infections produced by Bacillus coli have been treated by injections of vaccines made with this organism. The vaccine method of treatment has been found to be most successful in cases of chronic cystitis due to B. coli communis, although cholecystitis, appendix abscesses, endometritis and other local infections with this organism, have at times responded favorably to this method of treatment. The number of bacteria injected is seldom more than fifty million, usually smaller doses than this being used. Vaccines made from cultures isolated from the patient usually give the best results. The injections are repeated about every ten days.

Bacillus typhosus and Bacillus paratyphosus Infections. The typhoid bacillus group consist of several important members. Recognition of this fact has led to the use of vaccine made from cultures isolated from the patient to be treated. Vaccines made from killed cultures of typhoid bacilli have been used for immunization as well as for treatment of pathological conditions due to members of this group.

Pfeiffer and Kolle have made vaccines for immunization against typhoid fever from agar cultures, while in England the

vaccine usually is made from bouillon cultures. The organisms are killed by one hour's exposure to 53° C. to 60° C. Pfeiffer and Kolle inject sufficient amounts of vaccines to produce a local reaction lasting several days, and a general reaction, as is demonstrated by fever and malaise, lasting one to two days. Following the first injection, two to three injections of increasing amounts are made at intervals of eight days. The English method also produces marked disturbances at times. The duration of immunity conferred by this method of treatment varies, and is usually regarded as lasting from one to three years. The results of such immunization are still doubtful but statistics indicate that immunized individuals are less likely to contract the disease, and that when the disease is contracted it is less severe and less often fatal.

Typhoid bacillus vaccine has been tried in the treatment of typhoid fever. Here it has met with but little success. The best results with the vaccine have been obtained in cystitis, cholecystitis, and the local abscesses following attacks of typhoid fever. The doses injected range usually from five million to fifty million bacilli, the injections being repeated at intervals from eight to ten days. Autogenous vaccines usually give the best results.

Bacillus dysenteriae Infections. Vaccines made from dysentery bacilli have been used in prophylaxis as well as treatment of bacillary dysentery. Inasmuch as there are different strains of these bacilli, it is advisable to use vaccines made from the cultures isolated from the patient or with all of the different strains of B. dysenteriae.

Shiga has tried various methods of active immunization and has found that the best results are obtained either, by injecting subcutaneously anti-dysenteric serum and killed cultures of dysentery bacilli, or, by taking by mouth the killed cultures of this organism. By Shiga's method of simultaneous injection of anti-dysenteric serum and dysentery vaccine the death rate in Japan due to bacillary dysentery has been much reduced. The duration of immunity, according to Shiga, is about two months.

Vaccine treatment of dysentery can only be applied in certain cases. By the method of active immunization, immunity cannot be established in much less than eight days, and for this reason only subacute or chronic cases are amenable to treatment by vaccines. The number of cases treated by this method is still too

small to warrant a conclusion. It seems quite certain, however, that vaccine therapy may prove of value in this disease. It must be borne in mind that there are different strains which can produce the disease and that an autogenous vaccine will most likely give the best results. Usually fifty million bacilli are injected, the interval between injections being from eight to ten days.

Cholera Vaccines. Vaccines made from cholera vibrions have been used in protective immunization. Statistics on this method are still too meagre to warrant definite conclusions on its value.

Less common Infections. Vaccine treatment has been applied in most all infections produced by bacteria. The results obtained have varied, though Wright believes that the method is applicable in the treatment of all infections caused by bacteria.

SPECIFIC VACCINES FOR DISEASES OF UNKNOWN ETIOLOGY.

Rabies, Hydrophobia or Lyssa. This is a disease whose etiology is only indefinitely established. The organism responsible for the condition has never been grown. By some investigators, certain peculiar bodies, found in 1903 by Negri in the large nerve cells in the central nervous system, are regarded as the causal factors in the disease. Although these bodies are not universally accepted as the etiological factors, they are quite generally accepted as specific to this disease.

The inability to establish the etiology has not prevented the establishment of methods of immunization to this disease. The principle of the method of immunization most generally practised is based on the establishment of an active immunity during the period of incubation of the disease. In man the period of incubation usually lasts from four to six weeks, though it has been found to be as short as fifteen days and as long as one year. The relatively long period of incubation makes it possible to establish an active immunity before the symptoms of the disease develop.

Immunization is accomplished by repeated injections of so-called "fixed virus." Pasteur found that tissues and fluids from rabid animal vary considerably in virulence, and that the virulence of virus can be changed considerably. If successive re-inoculations into rabbits be made from the virus of a rabid dog the virus

is increased in virulence for rabbits. After a number of passages through rabbits the virus finally can no longer be exalted in virulence. This is then called the fixed virus, is usually obtained from the cord and kills a rabbit in six to seven days. This fixed virus can, by drying, be gradually decreased in virulence so that after fourteen days of drying it has lost all virulence for rabbits. To immunize the individual, virus of low virulence is first injected, after which injections are made with virus of greater virulence until at the end of the treatment injections of cord which has dried only from two to three days, are made.

At the present time there are in the United States many so-called "Pasteur Departments" for immunization against rabies. The particular technique employed in such departments varies in some details, still the fundamental and essential principles are the same.

In every case treated, an attempt is made to establish definitely the diagnosis of rabies. Two methods are employed to accomplish this: one is to examine for Negri bodies and the other is to make inoculations into rabbits. For both of these methods it is essential that the head of the rabid animal be preserved intact and sent to the laboratory for diagnosis. It is therefore recommended that rabid animals be killed by other methods than those which mutilate the head.

As soon as the diagnosis is established, or if this is impossible because the animal cannot be found, the patient should submit to treatment. This requires from two to four weeks and consists usually in daily injections of cord of hydrophobia rabbits, which has been aseptically dried over caustic potash at a temperature of about 23° C. for varying periods of time. After drying, a part of the cord is suspended in bouillon to which either 0.25 per cent carbolic acid or glycerine has been added as a preservative. The injections are made into the subcutaneous tissue and are usually not followed by much disturbance. If there is an inflammatory reaction, it is to be treated as any other cellulitis. During the treatment, the patient may go about his work, his bowels should be kept open and alcoholic excess avoided. Immunization is not fully attained until about two weeks after the last injection of virus.

Immunization is usually and preferably practiced at the hospital of Pasteur departments. The New York City Department of Health has also practised immunization by sending virus to physi-

cians outside of the City of New York. In such cases a stronger course of treatment is followed and an effort is made to limit this practice to places not more than twenty-four to thirty-six hours' distance from the laboratory.

The results of Pasteur treatment or active immunization against hydrophobia are most satisfactory. Without specific treatment the mortality in patients bitten by rabid dogs varies from 50 to 80 per cent, while in patients who have undergone a course of active immunization the death rate is about 0.5 per cent. To get good results, early treatment is essential, Bites on the face and head, and bites by mad wolves are not immunized against as successfully as bites by dogs in other parts of the body. After the symptoms of the disease have developed, active immunization can no longer be expected to be of value.

Immunization against rabies has also been attempted by the injection of anti-rabic serum. Babes and Lepp in 1889 found that they could protect dogs against rabies by injection of serum from dogs actively immunized to rabies. Tizzoni and Centanni have reported favorable results with anti-rabic serum. Marie has recently advocated the simultaneous injection of virus and anti-rabic serum. By this method it is possible to immunize against hydrophobia more rapidly. The value of these newer methods has not been proven so that at the present time conclusions as to their value are impossible.

Small-pox. Small-pox is a disease for which the etiological factor has not been determined, although Guanieri, Councilman, Calkins and others have found protozoan parasites in the lesions of the disease.

For a long time it has been known that one attack of small-pox will protect against subsequent contraction of the disease. This has led to the practice of acquiring immunity against severe forms of the disease by intentional inoculation and contraction of mild forms of the disease.

In 1796, Jenner inoculated a boy with virus from cow-pox on a dairy-maid's hand, and found that in this way cow-pox can be transmitted and immunity to small-pox be produced. Since then vaccination against small-pox by inoculation of cow-pox has been quite universally adopted. It is now accepted that cow-pox is a modified form of small-pox and that small-pox vaccination is a

process of active immunization by means of living organisms attenuated by passage through cows.

The methods of obtaining virus for vaccination vary. Up to 1870, virus was usually obtained from the pustule of the vaccinated individual. Now, however, vaccine is usually obtained from young calves which have been inoculated on the abdomen and thighs with vaccine from the pustules of healthy children or calves. The pustules on inoculated calves are cleaned and opened on about the third or fourth day and from the material contained in them either so-called "vaccine points" or "glycerinated virus" is made. The animals from which the vaccine is obtained are kept as clean as possible, asepsis and anti-sepsis being employed as much as is possible. When all precautions are taken the vaccine still contains many organisms.

Vaccine points are made by dipping sterile bone slips into the material from the pustule. The vaccine is then dried, kept under aseptic conditions and is ready for use. Glycerinated virus is made by softening the pustular material with glycerine. Glycerine has the additional advantage of killing many bacteria in the virus and makes it possible to put the virus up in tubes. Small-pox vaccine is seldom entirely sterile, the organisms present, however, in a well-prepared vaccine, consist of only few species and are usually harmless. After two to three weeks action of glycerine, vaccine is frequentily free from bacteria.

The durability of potency and efficiency of small-pox vaccine varies. Some vaccines lose their value in one month while others will remain efficient for three to four months. This emphasizes the importance of using fresh virus for vaccination against small-pox.

The method of vaccination varies. It is the custom now to produce scarifications of one-eighth to one-fourth of an inch in diameter on the well-cleaned arm or thigh. When vaccination is made on the arm, the area selected is about the point of insertion of the deltoid muscle. In cleaning the part where vaccination is to be made it is to be remembered that all antiseptics must be removed with sterile water before inoculation is made. Scarification is only made severe enough to produce an exudation of serum, bleeding being avoided as much as possible. After scarification,

vaccine on the point or from the tube is well rubbed in, the serum is allowed to dry and a dry sterile dressing is put on.

The reaction produced by successful vaccination usually appears after about three days. Locally, there is at first a papule, which becomes a pustule on the eighth or tenth day. About the end of the second week the vesicle changes to a scab, which comes off and leaves a scar. Constitutional symptoms usually appear about the third day and last until the end of the first week after vaccination. When there is infection, cellulitis and sloughing follow; these conditions are treated as any other infection, but usually ought not occur.

The duration of immunity varies and is uncertain. The longest time that immunity can be certainly relied upon is two years. The rule for vaccination which is generally adopted and advised consists in vaccination within the first or second year, certainly before entering school, re-vaccination within the tenth to fifteenth year and after that whenever there is an epidemic of small-pox or possibility of exposure to the disease.

The efficiency of vaccination against small-pox cannot be doubted. The mortality in vaccinated individuals is between five and eight per cent, while in the unvaccinated thirty-five to forty per cent of the cases terminate fatally. The disease thus is milder in vaccinated than in unvaccinated individuals. Moreover fewer cases of small-pox occur in the vaccinated than in those not vaccinated against small-pox.

FUTURE OF OPSONINS AND VACCINES.

As the method of determining the opsonic index stands today it is of questionable value in diagnosis and treatment of bacterial diseases. Opsonins may play no part in immunity; on the other hand, they may be quite important, and we be lacking in comprehension of their value and their determination. Further efforts should be made to study them.

The treatment of the bacterial diseases by killed cultures ought not be cast aside because of the unreliability of the present method of determining the opsonic index, inasmuch as opsonins are not the only immunizing substances produced by the injection

of bacterial vaccines. It seems that the injections of killed cultures have been both of prophylactic and curative value. The curative value is probably limited to the treatment of chronic diseases, because time is required to actively immunize. Moreover this method may be of especial value in the treatment of infections due to species that have many varieties, for here the individual may be immunized to the special organisms causing the disease. Injections of killed cultures will not cure all infections and bacterial diseases and in some cases many injections may be required to obtain the desired result.

Attention is again called to the fact there is no vaccine for boils, acne or any other condition, but there are vaccines for immunization against infections by the Mic. pyogenes aureus, albus or citreus, streptococci, typhoid bacilli, pneumococci, gonococci, etc. Vaccine therapy, to give good results, requires that in all infections treated the causal microörganisms or virus must be determined, before vaccines are resorted to.

Credit is to be given to Wright, not for the discovery of the method of immunization by injections of killed cultures, but for its revival. The employment of this method of inducing immunity and treating bacterial infections and diseases need not necessarily be associated with opsonins and opsonic immunity.

CHAPTER VIII.

SERUM THERAPY

In the discussion of theories of immunity, mention was made of the fact that in 1887 Fodor showed that the juices of the normal living body, especially the blood, possess bacteria-destroying property. Buchner, Behring, and Nuttall were among the first to coroborate the results of Fodor. It was later recognized that the cell-free blood serum of normal animals possesses properties of destroying bacteria. It is to be noted, however, that the blood of every species of animals will not destroy all species of bacteria. Furthermore, while in certain cases the degree of natural immunity corresponds to the amount of the bacteria-destroying substance present, still these two conditions are not always found in the same case. The substance which destroys bacteria, Buchner called "alexine."

In 1888 Hèricourt and Richet made the observation that blood serum from an animal immunized to Staphylococcus pyosepticus, confers when injected intraperitoneally into rabbits, an immunity to this organism. Babes and Lepp in 1889 reported the possibility of protecting animals against rabies by the injection of body fluid from animals immunized to rabies. However, these results were given but little attention until Behring and his pupils systematically investigated the subject.

Behring and Kitasato, in 1890, reported that mice can be immunized to tetanus by the injection of blood serum obtained from rabbits artificially immunized to tetanus. These investigators further reported that serum from tetanus-immune animals protects against the primary intoxication produced by the tetanus bacillus.

In 1888 Roux and Yersin discovered diphtheria toxin. Behring and Wernicke, in 1891, showed that blood serum from diph-

theria immune guinea pigs or rabbits possesses the ability, when injected intraperitoneally, to immunize normal guinea pigs against diphtheria and a diphtheria infection already in progress.

In 1892 these investigators further found that it is possible to immunize larger animals to diphtheria, and demonstrated in the blood of these animals protective and curative substances which can be transferred by the serum to other animals. The doses necessary to cure the disease were found to be larger than those required to immunize against the same. The amount of protective and curative substance produced was found to vary, to some extent at least, with the degree of active immunization of the animal from which the serum was obtained. It was further found by Behring that serum from animals immunized to diphtheria will protect against the toxin of Roux and Yersin.

Based on these observations, Behring has formulated a law which is generally known as "Behring's Law." According to this dictum, the blood or blood serum from an animal possessing **acquired** immunity is able to transmit this immunity to a susceptible individual or animal when this blood or blood serum is injected in the right amounts into the susceptible animal or individual. Individuals naturally immune to a particular infectious organism, do not possess in their blood or blood serum immunizing substances which can be transmitted to another individual. Immune substances which can be transmitted thus are not present naturally but must be produced by a process of immunization which consists either of undergoing the natural course of the disease or submitting to some method of artifical immunization. As a result of these investigations, there has arisen the practice of passive immunization, or, as it is more frequently called, serum therapy. The development of methods and success obtained by passive immunization against the action of tetanus and diphtheria bacilli, led to the belief that it would be a relatively easy matter to make specific sera against all disease-producing bacteria. It was soon found, however, that this is not possible. Acquired anti-toxic immunity has only a relatively limited application. Most pathogenic bacteria hold their special poison so firmly within the cell that the poison is only freed when the cell disintegrates, or when certain stimulating conditions as are found

in the body exist. Conditions favorable to the liberation of toxins have up to the present time been produced for a few bacterial species, so that most bacteria only liberate their toxin in the tissues of the body invaded. As a result of immunization to two widely different substances, toxin and bacterial cells, two different classes of immune sera have been manufactured. Of these two classes, the anti-toxic sera neutralize certain morbific bacterial products, while the anti-bacterial sera destroy the bacterial cells. It is not to be inferred from this that the same serum may not be both anti-toxic and anti-bacterial, for diphtheria toxin and cells, for example, may be used in immunization. Under such conditions the blood serum obtained will have both anti-toxic and anti-bacterial value.

Acquired anti-toxic immunity is an immunity due to a neutralization of toxin by a specific anti-toxin. The substance that is formed as a result of the combination of toxin and anti-toxin acts like a harmless and stable compound. Ehrlich, basing his observations on studies of the union of toxin and anti-toxin, classes the anti-toxic bodies formed with the anti-bodies of the first order, that is, the labile susbtance or complement takes no part in the reaction. In natural immunity to certain toxins the presence of specific anti-bodies cannot be demonstrated and under these conditions immunity is rather a non-susceptibility of the cells to the toxin. Metchnikoff has shown that tetanus toxin circulates freely in the blood of naturally immune animals. In certain other cases of natural immunity toxin combines with cells that are of little importance to the body or which are but little affected by the particular toxin. Acquired anti-toxic immunity thus differs from natural immunity in the formation of immune bodies. These immune bodies are found in the body fluids, especially the blood serum and can be injected into other animals or individuals and in this way confer upon them a passive immunity.

Acquired anti-bacterial immunity is an immunity dependent upon the rapid destruction of bacteria. The process is one of solution or lysis. The anti-bodies which produce lysis belong to Ehrlich's third order of immune bodies, that is, complement, which is easily destroyed by age, heat, etc., is necessary to bring about the solution of bacteria. Natural immunity to

certain bacteria is associated with the presence of lytic bodies. Behring found that blood serum from the white rat which is naturally immune to the action of anthrax bacilli, exerts a marked destructive effect on anthrax bacilli, while blood from animals not immune to anthrax bacilli exert no such destructive effects on these organisms.

On the other hand blood serum from the dog and the pigeon, both of which species are practically immune to the action of anthrax bacilli, posses no demonstrable bactericidal power. It is evident that natural immunity is not in all cases due to bactericidal action of blood serum of non-susceptible animals. Acquired anti-bacterial immunity differs from natural immunity to the action of bacteria, in that definite specific immune bodies are present. These immune bodies are found in the body fluids, especially the blood serum, and dissolve bacteria.

UNTOWARD EFFECTS OF SERUM INJECTIONS.

In the conference of passive immunity, blood and blood serum injections are made almost exclusively. Blood transfusions were made by Denis in 1667. While some good results were obtained, fever, embolli, bleeding, heamoglobinuria, and urticaria were sometimes produced by these blood transfusions. Up to 1894, before diphtheria anti-toxin was first generally used, injections of blood and blood serum were rather rare. Following the widespread practice of injection of diphtheria anti-toxin in blood, exanthemata followed in approximately twenty-two per cent of the cases. With the injection of diphtheria anti-toxin in blood serum, exanthemata were produced in only six or seven per cent of the cases. From time to time after 1894 cases have been reported in which injections of diphtheria anti-toxin were followed by skin manifestations. While the sequellæ of serum injections are usually not serious, still at times serious symptoms and even death have been reported as following injections of serum.

In 1905, v. Pirquet and Schick originated the term "Serum Krankheit," or serum disease. These investigators found that serum disease varies, two rather definite types of the disease being recognized; one type which results from the first injection of

serum and another type which follows the second and subsequent injections of serum.

Serum disease following the first injection of serum manifests itself after an incubation period of eight to twelve days, and is largely independent of the amount of serum injected. Of the symptoms of this disease, fever is the most constant, lasting usually during the entire course of the disease. The height of fever, however, is not an indicator of the disease, but from the curve of the temperature a prognosis can be made, because the temperature drops by lysis. Together with fever there is usually an exanthema, appearing most frequently as an urticaria. This appears first about the point of injection, later it is distributed symmetrically and usually itches severely. At times there is swelling of the glands, especially of the glands found in the region injected. The symptoms of pain in the glands are of prognostic value, in as much as the pain usually disappears or abates before the size of the gland decreases. During the period of incubation the leucocytes are increased in number, but during the height of the disease the number of leucocytes is markedly diminished. Joint pains are found in a small percentage of the cases of serum disease. The metacarpal, hand and knee joints, are most frequently effected. Usually these pains do not last long. In certain cases there is edema, but albuminuria is seldom or never present. The mucous membrane is seldom effected, which distinguishes the condition from scarlet fever and measles. The disease is further distinguished from measles by the absence of Koplick spots, coryza, conjunctivitis and increased efflorescence about the point of injection. From scarlet fever the condition is distinguished by its non-communicability by contact, and absence of scaling, nephritis and angina.

The disease following the second and subsequent injections of serum varies somewhat with the interval between injections. If an injection of serum is made from twelve to forty days after a preceding one, the incubation period is very short, the disease appearing at times in less than one hour after injection. If the interval between injections is from forty days to six months, there may be an immediate reaction, or else an immediate reaction with another reaction six to eight days later. If the interval between injections is over six months there is usu-

ally no immediate reaction, the symptoms appearing six to twelve days after injection. When the interval between injections is six days or less, the disease is not produced. Serum disease follows re-injections more frequently than it does in the first injection and is usually produced by smaller amounts of serum.

The "immediate reaction" occuring when re-injection is made within an interval of twelve to forty days, manifests itself in one to six hours after injection and usually reaches its maximum within twenty-four hours after injection. The "second reaction" is not seen after the first injection of serum but occurs most frequently when the interval between injections is between forty days and six months. The incubation period for the second reaction is somewhat shorter than it is for serum disease produced by first injections. The symptoms of serum disease produced by re-injection are usually more acute and general but of shorter duration. In many cases there is vomiting.

When serum disease was first recognized it was supposed to be caused by toxin in the immune serum. However, as early as 1894, Heubner expressed doubt as to the importance of specific immune sera in the production of the disease. Later the manifestations of serum disease were produced by the injection of normal serum. In 1906 Rosenau and Anderson of the Hygienic Laboratory of the Public Health and Marine Hospital Service of the United States, published a work in which experiments were reported on "sudden death" of guinea pigs following serum injections. These investigators found that re-injections of normal horse serum into guinea pigs are very poisonous if the interval between injections is more than ten days. The length of time for which this hypersusceptibility persists has not been definitely determined but lasts at least as long as two years and two days. On the other hand when the interval between injections is less than ten days, normal horse serum will produce no such effect.

The nature and causes of the reaction have been matters of considerable investigation and contention. The reaction was at first regarded as one resulting from the injection of toxin or poison. The long period of incubation, however, is explained with difficulty by such a conception of the phenomenon. While it is true that to produce disease by certain toxins, a period of incubation is necessary, this period is seldom as long as eight to twelve days.

It can hardly be conceived that horse serum has the ability to increase its amount of poison as can bacteria and other organisms capable of self reproduction. Rosenau and Anderson have determined quite definitely that the reaction is due to a difference in the susceptibility of individuals and not the toxicity of the serum.

v. Pirquet and Schick conclude that the phenomenon is due to the presence of specific anti-bodies. According to these investigators the anti-bodies are not precipitins, for man does not produce the precipitins readily with horse serum—in children three weeks are required before the precipitins can be detected and are found in the blood only from four to nine weeks. The "immediate reaction" which occurs when the second injection of horse serum is made twelve to forty days after the first, Pirquet and Schick explain as being due to the already present serum anti-bodies which combine with the immunizing substance in serum and produce poisonous substances, which in turn produce the disease.

Gay and Southard, in 1907, reported experiments which indicate that the theory of Pirquet and Schick is untenable. These investigators believe that "sudden death" in guinea pigs "sensitized to horse serum" is due to a substance they call "anaphylactine." This substance is found in normal horse serum, is not absorbed by the tissues of the guinea pig and is eliminated slowly. The anaphylactine in the guinea pig increases the avidity in the cells of the guinea pig, so that when more serum is injected the cells are "overwhelmed in the exercise of their eliminating functions, and functional equilibrium is so disturbed that local or general death may follow." Rosenau and Anderson had shown that the hypersusceptibility of the guinea pig for a certain protein is manifested upon a second injection of the corresponding protein. Later they showed that the substance anaphylactine is specific in the same sense. These investigators could not demonstrate this substance in the blood of the sensitized guinea pig during the incubation period, nor at any time in the blood serum of man, monkeys and cats.

The investigations on the phenomenon of hypersusceptibility of guinea pigs to horse serum in connection with serum disease in man, has lead to a considerable advancement in the methods

of administration of specific immune sera. While anaphylactine has not been found in the blood serum of man, later investigation may show that the phenomenon of serum disease in man corresponds to sudden death and anaphylaxis in guinea pigs.

The markedly beneficial results of injections of specific immune sera in the prevention and cure of certain diseases, have so far outweighed the undesirable and serious results which have followed serum therapy, that most physicians have given little attention to the contra-indications of serum injection. As the indications and contra-indications for injection of immune serum are better understood by physicians fewer objections to serum therapy, scientifically applied, will be made. That serious objections to the injection of immune sera have some foundation, is evidenced by the fact that Rosenau and Anderson have been able to collect a considerable number of cases of sudden deaths in man after the injection of immune serum. It has been noticed from the first that in the cases of sudden death following the injection of diphtheria anti-toxin there is marked respiratory embarrassment. Rosenau and Anderson believe the essential lesion of serum anaphylaxis is localized in the respiratory centers. In collecting statistics on this subject these investigators have found two cases, "and also others have come to our notice," in which sudden death followed the injection of anti-toxin into asthmatics. From these observations Rosenau and Anderson conclude that "the knowledge of the fact that injection of horse serum into asthmatics may be attended with danger, should be considered in the use of anti-toxins."

While there are some cases in which the injection of horse serum has been followed by sudden death due to a hypersusceptibility to horse serum, most of the deaths have undoubtedly been due to other causes. Frequently physicians and laymen wrongly attribute undesirable conditions to the use of diphtheria anti-toxin and other immune sera. In the use of some immune sera, especially diphtheria anti-toxin, recovery from the disease is so rapid and early, that the physician and patient overestimate the physical condition of the patient, and as a result the patient is frequently allowed a certain amount of exercise. This at times is followed by unfavorable symptoms. The condition in these cases is probably due to the action of diphtheria toxin rather than

of diphtheria anti-toxin. The heart paralysis which develops in some cases is probably only observed because by the use of diphtheria anti-toxin the patient remains alive long enough so that the heart paralysis may be observed. The action of diphtheria toxin on the heart and other organs has been known for some time and in serum laboratories it is not an unusual occurence to have an apparently well horse, which is undergoing immunization to the diphtheria bacillus and its toxin, die very suddenly after exercise. Post diphtheritic paralyses have been observed long before the introduction of the use of diphtheria anti-toxin, and to attribute this condition to anti-toxic serum is to attribute a symptom to a remedy, where the symptom is really one found in the disease treated by the remedy, diphtheria anti-toxin. In all cases treated with immune serum the patient ought to be kept quiet for some time after injection of serum, even though he has apparently recovered from the disease.

Following the good results obtained in the treatment of diphtheria by diphtheria anti-toxin and the protection against the disease conferred by the so-called immunizing dose of diphtheria anti-toxin, the serum has been used very extensively. In many cases, undoubtedly, serum has been used without proper indications. Many physicians have used diphtheria anti-toxin even though the diagnosis of diphtheria was not definitely made, because it was assumed that no harm could come from the use of the serum while great benefits might result if the condition were one of diphtheria. Some physicians have injected the immunizing dose fully as indiscriminately; whole families, attending physician and nurses have received immunizing doses of diphtheria anti-toxin. The indications for protective and curative injections of immune serum ought to rest on a better foundation. This is especially true because hypersusceptibility to serum, or serum disease, is more frequent following re-injections of serum. It is evident that serum ought only to be injected into the individual when certain indications are present.

Specific treatment with immune serum is only indicated when the diagnosis, and, in certain cases, the etiological factor has been determined. It frequently happens that a bacterialogical diagnosis cannot be made immediately. Under such conditions the clinician is warranted in using specific serum if there is such

clinical evidence as is usually found in infections caused by the corresponding organisms, or if the patient has been exposed to infection from a previously diagnosed case of the communicable disease.

When injections of immune sera are to be made frequently, and over long periods of time, as is the case with most anti-bacterial sera, the injections should be made at intervals of less than ten days, because, if the interval is longer than this, serum disease is more likely to develop.

The indications for injection of immunizing doses are not clear nor definite. Nurses and doctors, however, now rarely receive immunizing doses of immune serum. This is especially true of diphtheria anti-toxin. The reasons for the abandonment of the practice of immunizing doctors and nurses, are based on the fact that doctors and nurses are better able to avoid infection and so usually do not contract the diseases of their patients, and because the protection conferred by passive immunization is only of short duration. Doctors and nurses, because so frequently exposed to infectious diseases, would have to be injected frequently and at different intervals of time to be immune to the various infections of their patients. Serum injections at intervals of more than ten days, however, are more likely to give rise to serum disease.

The custom concerning immunization of individuals against possible infection because of exposure, varies. It is the rule of some physicians, upon the diagnosis of diphtheria in one member of the family, to give all members of the family an immunizing dose of diphtheria anti-toxin. In some of the large children's hospitals and clinics diphtheria anti-toxin is given to all patients on admission, this being done to avoid the outbreak of diphtheria epidemics. Such preventative measures hardly seem warranted. The custom of most physicians now is to give immunizing doses of some specific anti-sera to such members of a family or household as are most likely to contract the disease; thus for example, diphtheria anti-toxin is given only to the children in a home in which there is diphtheria. On this basis administration of anti-sera is justifiable, while indiscriminate immunization by the injection of serum is costly and may also sensitize individuals to serum, which might later make the

use of serum difficult or impossible even though injection of immune serum were definitely indicated. While it is true that some sera are ordinarily injected at intervals of two or three weeks without the production of serious results, still it frequently happens that such a degree of hypersensitization to serum develops that passive immunization must be given up entirely.

Since the introduction of serum therapy, largè numbers of persons have been injected with immune serum and great benefits have been derived from the use of different sera. The cases of serum sickness, especially the rashes, have been relatively frequent, yet are so far outweighed by the beneficial results that they must not prevent the physician from the use of immune sera when they are indicated. The cases of sudden death following injection of serum fortunately have been very rare. As further knowledge concerning the untoward effects of the injection of horse serum is gained, serum disease and sudden death will become less frequent. The essential points, which have so far been developed, indicate, that specific sera are to be used only after a definite diagnosis of the disease, that there is some danger attending the injection of horse serum into asthmatics, and that untoward effects of serum injections are more frequent when the interval between injections ranges from ten days to several years.

CONCENTRATION AND PURIFICATION OF SERUM.

Frequently large amounts of specific immune substances are to be injected in the treatment of infections. Hypodermic injection of large amounts of serum, however, is painful and more likely to produce serum disease, especially the rashes. These objectionable features of serum therapy have resulted in an attempt to produce serum of greater concentration and purity. It has been found that bacteria and toxins from certain cultures are more potent and cause animals to produce immune sera of higher protective value. As a result virulent cultures, or toxins produced by virulent cultures, have been used in active immunization of the animal that is to furnish the immune serum.

Attempts have been made to obtain an increased potency by separating the immune substance from the non-immune substance of specific serum. Dieudonné, in 1897, showed that the protieds precipitated from diphtheria anti-toxin by acetic and

carbonic acid, contain none of the anti-toxins. In this same year Belfanti and Carbone found that with the globulins precipitated by magnesium sulphate, diphtheria anti-toxin is thrown out. Atkinson, in 1901, and others since then, have shown that during immunization the serum-globulin content of serum increases. Gibson and Banzhaf, however, have found that the increase in serum-globulin is not necessarily associated with the accumulation of anti-toxin in the blood. Early in 1906 Gibson, working in the Research Laboratories of the Department of Health of New York City, reported practical methods for concentrating and purifying diphtheria anti-toxins. According to this method, by half saturation of anti-toxic serum with ammonnium sulphate, the globulins, nucleo-proteids, and similar substances are thrown out. These contain the immune bodies. The precipitate is again dissolved in a saturated solution of sodium chloride. Now only the anti-toxin fraction and some of the globulins are in solution. After filtration, acetic acid is added to the filtrate to precipitate the anti-toxic globulins which are then filtered off, dried with paper, dialyzed, neutralized and again dialized for several days. After dialysis the solution is made isotonic by the addition of sodium chloride. The sulution is then filtered through a Berkefeld filter to remove any bacteria that may be present and chloroform added as an antiseptic. This method has been attempted for the various immune sera, but up to the present time it is profitable only for the concentration of diphtheria and tetanus anti-toxins. Recently Banzhaf has found that more concentrated anti-toxin may be obtained by heating the serum to 57° for some hours before separating the anti-toxic globulins. By Gibson's method the concentration is increased from three to five times, while by Banzhaf's from eight to ten times.

By the use of concentrated and refined serum the constitutional disturbances and rashes are somewhat less frequent and not as severe. The principle advantage of the product obtained by these methods, however, lies in the fact that a large amount of immune substance can be injected in small amounts of material.

DRIED IMMUNE SERUM.

Relatively recently, immune sera, especially anti-diphtheritic and anti-tetanic sera, have been dried after concentration and refinement. It was mentioned earlier that when serum is dried

soon after being drawn, the immune body and especially the complement are preserved longer. Among the advantages of dried immune sera may be mentioned: the lack of deterioration, the cheapness of manufacture; the convenience of carrying about in the medicine case; and the possible administration of immune substances by the mouth. Undoubtedly these products will prove to be of considerable value and are not to be lost sight of.

ORAL ADMINISTRATION OF IMMUNE SERUM.

In 1892 Ehrlich discovered that ricin anti-toxins can be absorbed by the intestinal canal. In 1893 Wernicke, working with Behring, established the fact that anti-toxic substances in the body fluids of diphtheria-immune animals are absorbed by the digestive tract; thus, dogs fed on the meat of diphtheria-immune sheep obtain some immunity to diphtheria. The possibility of oral administration of anti-toxic sera has been recently advanced by McClintock and King. These investigators have found that the best results by oral administration of diphtheria anti-toxin are obtained by the following method: "One half-hour before administering the serum the child is given one glass of one per cent sodium bicarbonate solution. When the anti-toxin is given there is added one minim Fl. Ext. Opii and from four to ten minims of saturated solution of salol in chloroform. When possible no food should be given for at least four hours before administering the serum." This method is adopted to inhibit the digestion of the anti-toxin so as to make the absorption of the unchanged anti-toxin possible. Dried diphtheria anti-toxic globulins were found to give satisfactory results. By oral administration of diphtheria anti-toxin neither serum sickness nor anaphylaxis was observed. At present this method is still of questionable value and requires further investigation.

ANTI-TOXIC SERA.

It was stated earlier that sera containing bodies which neutralized toxin are called anti-toxic sera. The immune bodies are receptors of the first order of Ehrlich (see p. 16), which have been thrown off as a result of overproduction of certain receptors.

The overproduction of these receptors occurs as a result of the presence of specific toxins, found in infections with specific organisms, or after experimental injection of certain toxins. As is the case with other anti-bodies, anti-toxins are present to some degree in many normal individuals, thus diphtheria anti-toxin was found by Bolton to be present in the blood serum of 30 per cent of the horses examined, and in 50 per cent of the children and 81 per cent of the adults examined by Wasserman. Various substances have been used to produce an increase of specific anti-toxins and as a result a considerable number of anti-toxins have been made. Among these may be mentioned the anti-toxins which combine with the toxins of the organisms of diphtheria, tetanus, pyocyaneaus, symptomatic anthrax, and botulism, with snake and spider venoms, with ricin, aprin and robin, and with the glucosids of toad stools and poison ivy. Of the anti-toxic sera, the anti-diphtheritic and anti-tetanic have been of most importance.

In the treatment of diseases for which anti-toxic sera have been made, it must always be remembered that anti-toxin neutralizes prepared toxin and combination can take place only when the receptors of the toxins and anti-toxins are free. As soon as the cells and toxins have combined and the body cells have been injured as a consequence, no amount of anti-toxin can protect the cell from the action of the poison molecule. Anti-toxic bodies can only anchor and render inert or harmless such toxins as are free or have uncombined receptors. This emphasizes the importance of the early administration of anti-toxic serum because at this time the poison molecules can be anchored by the anti-toxic substances instead of combining with the cells of the body.

DIPHTHERIA ANTI-TOXIN.

Ferran, early in 1890, and Fraenckel and Behring later in the same year, published methods by which experimental animals can be immunized to diphtheria. Behring and Kitashima, in 1891, published methods for the immunization of guinea pigs to diphtheria toxin. In 1892 Behring and Wernicke emphasized the presence of protective substances in serum of diphtheria-immune animals. Serum therapy as applied to the treatment of

diphtheria in man began in 1891 and 1892. The first diphtheria anti-toxin was put on the market in August, 1894. Since then diphtheria anti-toxin has been used extensively.

The method for making diphtheria anti-toxin has gone through various stages of development. All diphtheria anti-toxin used in the United States is made practically after the same method, which is as follows: Young, vigorous and absolutely healthy horses are immunized, although other species of animals have also been used. During immunization the animal receives repeated injections of diphtheria toxin. This toxin is obtained by growing a virulent culture of B. diphtheria on the surface of beef bouillon in an Ehrlenmeyer flask. After one week's growth at a temperature of 35° to 36° C., the culture is rendered sterile by adding carbolic acid in amounts to make a 0.5 per cent carbolic acid solution. After 48 hours the diphtheria bacilli have settled to the bottom, when the bouillon is filtered through a Berkefeld filter. The germ-free bouillon contains the diphtheria toxin and ought to be potent enough so that 0.01 c. c. will kill a 250 gram guinea pig on or before the fourth day after subcutaneous injection. The horse or animal to be immunized receives enough of this toxin in bouillon to kill five thousand guinea pigs of 250 grams each. At the time this amount of toxin is injected the horse also receives an injection of ten thousand units of diphtheria anti-toxin. After three to five days, when the fever has subsided, a somewhat larger dose of toxin and the same amount of anti-toxin are injected. A third injection is made after another interval of from three to five days. After this usually no more anti-toxin is injected with the toxin, the doses of which are constantly increased and injected at intervals of from five to eight days. After about two months of treatment, if the immunization has been successful, the horse will usually tolerate enough toxin at one injection to kill one hundred thousand guinea pigs of 250 grams each.

At the end of six weeks to two months, samples of blood are drawn and tested as to protective value. If the anti-toxic value is high, the horse is usually bled to death by tapping the jugular vein. The withdrawal of serum is made under aseptic conditions. The bottles containing the blood are slanted and after four to five days the serum is drawn off.

By means of Gibson's or Banzhaf's method the serum is now concentrated and refined. After this it is standardized, i. e., its strength is determined. The United States government determines the unit of diphtheria anti-toxin in the United States, and the Government requires that all diphtheria anti-toxin made by manufacturers, having a United States government license, must conform to this standard. This emphasizes the importance of using only diphtheria anti-toxin made by manufacturers having a United States Government license. Because the toxic value of diphtheria toxin changes with ageing and other conditions, the Hygienic Laboratory of the U. S. Public Health and Marine Hospital Service, issues from time to time standard anti-toxic serum. With this serum the strength of the toxin used in determining the anti-toxic value of serum is gauged. The "immunity unit," or unit of anti-toxin, is the amount of diphtheria anti-toxic serum which will just neutralize one hundred times the smallest amount of toxin necessary to kill a 250 gram guinea pig in four days. The anti-toxic value of serum is determined by mixing one hundred times the smallest fatal dose of fresh diphtheria toxin with varying amounts of the diphtheria anti-toxin, allowing the mixtures to stand for fifteen minutes and then injecting them into 250 gram guinea pigs. The smallest amount of serum which will protect a 250 gram guinea pig for more than four days against one hundred times the smallest amount of diphtheria toxin necessary to kill a 250 gram guinea pig, contains one unit of diphtheria anti-toxin.

At the time of bleeding immunized horses, that is after six to eight weeks of immunization, the serum may contain from one hundred to one thousand units of diphtheria anti-toxin per c. c. The concentrated and refined diphtheria anti-toxin contains usually from three hundred to two thousand units of anti-toxin per c. c. The various manufacturers of diphtheria anti-toxin now furnish their product in syringes containing a determined number of units of anti-toxin. These packages are stamped so as to indicate the date after which they can be exchanged, free of charge, for more recently tested serum. Because of the degeneration in potency of diphtheria anti-toxin in the fluid condition, it is desirable that no sera be used after the date when they are to be exchanged for new sera.

The therapeutic application of diphtheria anti-toxin has given most satisfactory results, and is now recognized and regarded as the **specific** for diphtheria. The statistics on the death rate from diphtheria have been collected at many different times, and all indicate that, while before diphtheria anti-toxin was used over fifty per cent of the cases resulted fatally, since the application of this method of treatment, the death rate from diphtheria has fallen below twenty per cent, and according to some statistics to five per cent. In Boston the death rate of diphtheria from 1888 to 1894 was 43.2 per cent, while from 1895, after which diphtheria anti-toxin was used extensively, to 1904, inclusive, the death rate has fallen to 11.84 per cent. Still better results will probably be obtained as this method of treatment becomes more universal and is better understood.

The method of treatment of diphtheria with diphtheria anti-toxin varies. However, some definite principles of treatment have been established. It is very essential that all cases be **treated** as **early** as possible. The reasons for this are clear when it is understood that as toxin is liberated by diphtheria bacilli, it tends to combine with the cells of the body. After this union has once taken place the cell is rapidly injured. In order that diphtheria anti-toxin combine with these toxins it is essential that the immune substances be present at the time of the liberation of the toxin. Most statistics show that in ordinary cases of diptheria the death rate is **nil** when diphtheria anti-toxin is injected on the first day of the disease. Even when treated with diphtheria anti-toxin on the second day, the death rate is below five per cent; however, when specific treatment is not instituted until after the fourth day, twenty per cent of the patients do not recover.

The **dose** to be given in the different conditions varies, and no hard and firm rules can be laid down. There are, however, some quite generally accepted rules as to doses.

The ordinary case of diphtheria, treated on the first day of the disease, receives injections of from two to three thousand units of anti-toxin. When the serum is not given until the second day, usually three to four thousand units are injected.

In the pharyngeal and laryngeal types of diphtheria larger doses are usually given—seldom less than five thousand units.

When the disease comes under observation late or is very severe twenty thousand to one hundred thousand units may be necessary. Evidence has been obtained that toxin may be taken from the cells with which it has combined when very large doses of anti-toxin are injected. No case of diphtheria should be regarded as being too severe or too far advanced to be treated by diphtheria anti-toxin, but in such cases large doses should be given, as small doses are of no avail, because they do not neutralize all the toxin present. The age of the patient, unless very young, should have no influence on the amount of anti-toxin injected.

Diphtheria anti-toxin, as any other therapeutic agent, should be given in sufficient quantities to accomplish the full **therapeutic action.** To determine this the effects of diphtheria anti-toxin must be recognized. The results of injections of sufficient amounts of anti-toxin are: general improvement of the patient's condition, reduction of fever, improvement of the pulse, but most noticeable of all is the shriveling of the membrane, decrease of discharge, and less fetid odor of the breath. When these effects do not appear from six to eight hours after injection, more diphtheria anti-toxin should be injected. Re-injections usually are larger than first injections, and are indicated at any time when the patient's condition becomes more grave, or when improvement does not come in six to eight hours after injection. At times diphtheria anti-toxin does not give satisfactory results in the treatment of diphtheria. In most of these cases, however, the treatment is begun too late or there is infection with other organisms. Of these organisms streptococci are most important and are responsible for many of the fatal cases of diphtheria. Such cases are at times treated with both diphtheria anti-toxin and anti-streptococci serum.

Various attempts have been made to administer diphtheria anti-toxin by the mouth. The work of McClintock and King on this method has already been referred to. Before oral administration of diphtheria anti-toxin can be generally employed, more experimentation will be necessary.

The use of dried diphtheria anti-toxic globulins, which are dissolved in salt solution before injection, is relatively recent. If the results obtained by their use are as favorable as has been reported, it may be expected that they will be readily taken up

by the medical profession. Serum, however, has the advantage of always being ready for use and not requiring sterilization before administration.

In some of the severer cases of nasal diphtheria, anti-toxin has been sprayed on the membrane with some beneficial results. Frequently too, it is observed that diphtheria bacilli remains viable in the nose and throat after an apparent recovery from the disease. Such persons frequently are the cause of the spread of diphtheria. Various attempts have been made to remove the diphtheria bacilli from the nose and throat of these apparently well individuals. In addition to the antiseptic methods employed in the treatment of this condition, diphtheria anti-toxin has been applied locally. Recently it has been proposed to actively immunize such individuals by the injection of bacterial vaccines made from the cultures of diphtheria bacilli isolated from the patient.

Immunization of well individuals against possible diphtheria infection has been practiced for some time. It has been quite definitely proven that injection of a relatively small number of anti-toxic units will protect from four to six weeks against infection with the diphtheria bacillus. The doses generally recommended are from three hundred to five hundred units in small children and one thousand units for older children and adults. While the custom concerning the immunization of well persons varies, it is quite generally accepted that children who have been exposed to diphtheria should receive at once immunizing doses of diphtheria, while adults seldom are treated in this way.

The definitely beneficial results which have been obtained by the use of diphtheria anti-toxin in the treatment of diphtheria, ought to be sufficient to convince the practitioner that diphtheria anti-toxin should be used in practically all cases of diphtheria. Moreover the practioner ought to know enough concerning the symptoms and complications of diphtheria, and the untoward effects of serum injection to distinguish the conditions dependent upon the disease and those dependent upon the serum injected. It is not unusual for the practioner to diagnose the transient serum rashes as erysiplas, and the edema, following serum injections,

as those due to Bright's disease. Practioners are undoubtedly largely responsible for the misconceptions of laymen concerning the effects of diphtheria anti-toxin injections. As a result of these misconceptions frequently consent to use diphtheria anti-toxin, when it is definitely indicated, cannot be obtained, and yearly numbers of children whose lives could undoubtedly have been saved are carried to the grave.

TETANUS ANTI-TOXIN.

In 1890, Behring and Kitasato immunized mice and rabbits to tetanus by injecting cultures of the bacillus of tetanus. These investigators found that blood from rabbits immunized to tetanus bacilli, is able to protect mice against tetanus. The first reliable anti-tetanic serum to be used in man, was put onto the market in 1896.

The process of producing anti-tetanic serum, is similar to that employed to produce anti-diphtheria serum—blood is drawn from horses that have received injection of increasing amounts of tetanus toxin. The toxin injected is produced by growing the tetanus bacillus on bouillon. After ten to fifteen days of incubation under anacrobic conditions, the bouillon culture is filtered through a Berkefeld filter. The germ free filtrate contains the toxin, which is present usually in large amounts. At the first injection into the horse usually one-half c. c. of toxic bouillon, together with anti-tetanic serum are injected. The amount of toxin is increased at each injection, until finally seven hundred to eight hundred c. c. of toxin are tolerated when given in one injection. After the third injection the anti-tetanic serum is usually omitted. After several months of treatment, and complete recovery from the last injection, the horses are bled and the serum is collected. The serum is then concentrated and refined after the same methods that are used for diphtheria anti-toxin.

The standardization of tetanus anti-toxin until very recently has been indefinite and unsatisfactory. At the present time different standards exist in the various countries. The unit of anti-toxin for tetanus for the United States has been fixed as that amount of tetanus anti-toxin that will protect a 350 gram guinea pig for ninety-six hours against one thousand times the smallest fatal dose of tetanus toxin. In order that the standard may be

the same throughout the United States the Hygienic Laboratory of the United States Public Health and Marine Hospital Service sends out at regular intervals a stable precipitated tetanus toxin which is called the "test dose" and contains one hundred times the smallest fatal dose. It will be observed that a unit of tetanus anti-toxin contains more than ten times as much neutralizing value as does a unit of diphtheria anti-toxin. The various anti-tetanic serum producers furnish their product in suitable syringes, and, as is the case with diphtheria anti-toxin, state the date after which it is desirable that the serum be returned if not used.

The value of tetanus anti-toxin and the methods for administration can only be judged after study of the action of the tetanus bacillus and its toxin. Tetanus in man is usually the result of the subcutaneous introduction of tetanus bacilli, some foreign matter and usually saprophytic organisms. Thus the disease follows most frequently upon puncture and contused wounds as the result of injuries made by machinery, pistol shots, traumatism especially in stablemen, infection of the naval during or after birth, and sometimes as a result of the injection of contaminated vaccines and sera. The distribution of the organism is very wide, being found practically everywhere where there is animal habitation.

After the entrance of the tetanus bacillus, usually there is little change produced in the tissues infected. Suppuration occurs practically only as a result of other organisms. Usually the organisms remain localized. The disease and its symptoms are entirely dependent on the absorption of the toxins produced by the tetanus bacilli. Tetanus toxin circulates in the blood and lymph, but shows no symptoms until it unites with and is absorbed by the end organs of the motor nerves and the central nervous system. To reach the end organs the toxin is carried through the axis cylinders. It is thus seen that when the symptoms of the disease appear, the toxins have already combined with the cells, are exerting their toxic effects on them and can no longer combine with the anti-toxin which is not taken up by the central nervous system. In cases not treated with tetanus anti-toxin, Rose has found that ninety-one per cent of the cases in which the incubation period is short, terminate fatally; 81.3 per cent of those

having a medium incubation period die, while of the cases with a long incubation period, 52.9 per cent of the patients die.

Tetanus anti-toxin can only bind tetanus toxin before it is taken up by the nerve cells and axis cylinder. Frequently, however, the disease is not suspected until after the symptoms appear. Because of this the value of tetanus anti-toxin lies mainly in its **prophylactic** application. As a preventative of tetanus, tetanus anti-toxin has proven to be of great value. However to obtain beneficial results injections should be made early, as soon after infection as is possible, and inasmuch as immunity lasts only two to three weeks, the injections should be repeated as long as danger of infection exists. The immunizing dose which is generally used consists of fifteen hundred immunity units. The wounds after which immunizing doses of tetanus anti-toxin are to be used are those made by burns with toy pistols, fire-works, pistol shot wounds, injuries occuring while working about the stable or infected with fertilized garden earth or manure. The value of this method cannot be definitely determined, for if tetanus anti-toxin is used successfully, it is difficult to decide whether or not there has been infection with the tetanus bacillus. It is the belief of some, however, that tetanus can be prevented entirely if after all injuries of the kind likely to be infected with tetanus bacilli immunizing doses of tetanus anti-toxin are injected early. Jordan states that in the United States in 1903, there were 4,449 Fourth of July injuries, of which 406 cases died of tetanus; while in 1907, when tetanus anti-toxin was used quite universally for such wounds, there were only sixty-two deaths caused by tetanus, following 4,413 Fourth of July injuries.

The use of tetanus anti-toxin as a **curative** measure has not given universal success. This is undoubtedly due to the fact that after the symptoms appear the tetanus toxin has already combined with and injured the nerve cells, and is no longer amenable to neutralization with tetanus anti-toxin. It is usually conceded that if tetanus anti-toxin is not given within thirty hours after the appearance of symptoms, subcutaneous injections of anti-toxins is of little avail. When the disease has advanced, the large nerves in the vicinity of the infection and the spinal canal have been injected. The therapeutic dose of tetanus anti-toxin administered varies. If it is a case of short incubation, that

is eight days or less, injections of from 15,000 to 20,000 units are made every two to eight hours until there is abatement of the symptoms. These cases usually terminate fatally, even when tetanus anti-toxin is given. The cases where the incubation period is longer are more frequently recovered from. In all cases early injections in large doses are indicated when the symptoms have appeared, and radical methods of injection are justifiable, because most cases without anti-toxin terminate fatally. As a result of tetanus anti-toxin, the death rate in animals as determined by Behring has been found to be reduced from 88 per cent to 40 or 45 per cent. The curative value of tetanus anti-toxin in man cannot be well demonstrated by statistics. While the results are not nearly as favorable as those obtained by treating diphtheria with diphtheria anti-toxin, still tetanus anti-toxin ought to be used in all cases of tetanus.

Relatively recently a dried tetanus anti-toxin has been made. This powder is used principally as a dressing for wounds which are likely to be injected, but it may also be used for injection after dissolving in salt solution. For the latter use it has the objection that it dissolves with difficulty. While relatively little is known concerning it, it has advantages similar to those of dried diphtheria anti-toxin and may prove of considerable value to the medical profession.

As a result of the decreased number of cases of tetanus following Fourth of July wounds, when immunizing doses of tetanus anti-toxin are given, the physician should in all cases of such injury administer to the patient an immunizing dose of tetanus anti-toxin. Moreover all wounds in which tetanus infection might occur, should be thoroughly cleansed and the patient immunized with tetanus anti-toxin. When symptoms of tetanus have developed the patient ought to have every advantage of any benefits that may be derived from the use of large and repeated injections of tetanus anti-toxin.

ANTI-BACTERIAL SERA.

Sera containing substances which result in the death and destruction of bacteria are called anti-bacterial sera. Acquired anti-bacterial immunity depends on destruction of bacteria be-

fore they have had sufficient chance for multiplication and production of poisons which kill the cells in the body. For this reason anti-bacterial sera are frequently called bactericidal sera. In the serum of individuals and animals that have acquired anti-bacterial immunity, various kinds of anti-bodies are found. The best known of these bodies are bacteriolysins, opsonins, precipitins and agglutinins. These prepared anti-bodies are introduced into the body in passive immunization. The importance and value in immunity of all of these anti-bodies is not known, but it is generally accepted that agglutinins and precipitins are of little or no value, while opsonins and bacteriolysins are considered of importance in anti-bacterial immunity. Bacteriolytic immune sera depend for their action on specific substances called "bacteriolysins," which dissolve bacteria. The value of opsonins in bactericidal sera to be used in injections for the purposes of conferring passive immunity, is still indefinitely understood, although some investigators have attached considerable importance to their presence in sera.

The anti-bodies in anti-sera called agglutinins and precipitins belong to the second order of receptors, the bacteriolysins to the third order, while in regard to opsonins, no statement can be made at the present time—some regarding them as belonging to the second and others to the third order of receptors.

Anti-sera containing receptors of either the second or third order are easily inactivated by heat, age, acids, etc. After inactivation they are no longer able to produce agglutination, precipitation, lysis, or opsonification. The loss of power to produce these effects depends on the destruction of ferment-like substances, which are a part of the agglutinin and precipitin receptors and are furnished to the receptors of the third order by the fresh blood serum. (See page 18.) Inactivated bacteriolytic serum differs from inactivated or aged agglutinating or precipitating serum in that on the addition of fresh serum the anti-bodies producing lysis can be re-activated, while the agglutinating and precipitating properties cannot be restored in this way. Because the different specific bacteriolytic sera used in passive immunization are usually not freshly drawn and therefore have been inactivated by age, the individual or animal immunized must furnish the complement or ferment-like substance so as to make the destruction of bacteria

possible. The normal individual in order to utilize all the lytic and possibly the opsonic anti-body in a specific serum, therefore must possess large amounts of complement. Numerous investigators, among which may be mentioned Ehrlich, Morgenroth and Metchnikoff, have found that in certain diseased conditions there is an actual decrease in complement. Moreover, as far as can be determined, complement is not increased during disease and immunization. Richardson has shown that the serum of typhoid patients is not usually able to destroy typhoid bacilli, but that upon addition of fresh serum from a normal individual the typhoid immune serum is able to bring about complete lysis of typhoid bacilli. Numerous similar observations have been made in natural infections in man. In the efficiency of anti-bacterial sera the presence of sufficient amounts of complement undoubtedly plays a large part and in certain cases the lack of complement probably is the cause of failure of beneficial action of certain specific anti-bacterial serum. Various attempts have been made to increase the amount of complement. Anti-complement has been used to immunize in order to excite the over-production of complement. Injection of fresh complement with the immune serum has been tried by some investigators. Efforts have been made to preserve the complement in immune sera by freezing the serum and keeping it cold until it is injected. Recently dried immune sera have been advocated because it is known that complement is preserved for a long time in serum dried down soon after it is drawn. It is questionable, however, whether by any of these methods enough complement will be present to activate the amount of immune bodies injected. Hiss recently has published results on the curative action of subcutaneous and intraperitoneal injection of extracts of leucocytes from normal rabbits. Of this leucotic extract Hiss says, "The action of the leucocytic extract may be due to the enhancement of the bacteriolytic action of the animal's plasma by the introduction of complement—but is most likely chiefly due to the poison neutralizing or destroying bodies."

The practical application of specific anti-bacterial sera in the treatment of the various infections in man has been tried on a large scale. It has been found that by the early injection of serum from animals actively immunized to various organisms, animals can be rendered non-susceptible to infections with pneu-

mococci, gonococci, streptococci, typhoid and dysentery bacilli, cholera spirillae and many other microörganisms. Based on these results, many different anti-bacterial sera have been used in passive immunization in man. The results of anti-bacterial sera in the treatment of diseases of man have, however, not been as satisfactory as those obtained with specific anti-toxic sera. The importance of complement has already been emphasized. There are, however, other reasons why anti-bacterial sera have not yielded as good results as anti-toxic sera.

Anti-bacterial substances, with the aid of complement, have the power of destroying bacteria but not of neutralizing bacterial toxins. For this reason it is essential that anti-bacterial sera be used early enough in the disease, before the bacteria have had a chance to multiply sufficiently and to set free enough poison to injure and kill the cells. Early injection of specific sera unfortunately is not possible in many of the diseases for which specific anti-bacterial sera have been used, but as our methods of early diagnosis are improved earlier administration of these sera will be made. It is to be remembered that it is of the utmost importance that these sera be used before the infecting organisms have multiplied greatly.

Another reason of the failure of anti-bacterial sera to cure disease is dependent upon the fact that as the bacteria are dissolved and destroyed by the action of immune substance and complement, the intracellular toxins are liberated. If the body cells are not able to cope with the increased amount of toxin liberated upon the solution of the bacteria, they will die and the gravity of the disease be increased. Hiss has produced evidence to show that the leucocytes contain substances neutralizing these poisons. To have liberated the minimum amount of toxin, anti-bacterial sera should be administered when the number of invading bacteria is relatively small, which will be early in the disease.

Anti-bacterial sera are standardized with more difficulty and less reliably than are the anti-toxic sera. Attempts have been made to determine the number of smallest fatal doses of bacteria a given amount of serum will protect against. It is to be remembered, however, that with living microörganisms, a multiple of the fatal dose is not as much more severe than a single dose as

the multiple would indicate. Attempts have also been made to determine the protective value of specific anti-bacterial serum by injecting varying amounts of sera, and bacteria, which have been mixed in vitrio and allowed to act for some time before injection. The value of some of the lytic serum, as determined by this method, is high. Frequently it is not possible to test the bactericidal power when, as a result of the difficulties of determining the amounts of specific bactericidal substances in the serum, the agglutinating and precipitating values are determined. This method of standardization is not followed because agglutinins and precipitins are regarded as bactericidal substances but because these anti-bodies, to some extent at least, accompany bactericidal power.

As was stated earlier, specific anti-bacterial sera have been made by injections of practically all the disease producing bacteria. The most important of these are the sera which contain specific substances by which they destroy streptococci, meningococci, pneunococci, typhoid and dysentary bacilli, and staphlycocci.

ANTI-STREPTOCOCCIC SERUM.

Anti-streptococcic serum was first made bv Marmorek, in 1895. This investigator immunized horses by injections of increasing doses of living virulent streptococci. With serum from these animals he was able to confer passive immunity to rabbits and also made attempts to treat erysipelas and pueperal fever in the human. Denys and LeClef have immunized animals with bouillon cultures of streptococci and claim to get favorable results upon injection of serum from the immunized animals. Van der Velde, realizing that there are different strains of streptococci, and that the anti-sera produced are specific for the especial strain used in immunization, immunized animals with the different strains of streptococcic, thus making what is called a "polyvalent" anti-streptococcic serum.

The sera which are now on the market are almost universally made by immunizing horses with repeated injections of increasing

doses of killed and later living bouillon cultures of numerous strains of streptococci recently isolated from patients. Several months are required to immunize horses from which the immune serum is to be obtained. After testing the serum for sterility it is usually tubed in suitable syringes.

The method of action of anti-streptococcic serum is little understood. The serum contains agglutinins but these have not been regarded of value in immunization. With the discovery of bacteriotropins by Neufeld and Rimpau and opsonins by Wright and Douglas new light has been thrown on the action of these sera, for it has been found that in anti-streptococcic serum there is present a specific substance which makes streptococci vulnerable to ingestion by leucocytes.

Standardization and determination of the amount of protective substances in anti-streptococcic serum has not been successful, because the susceptibility of animals to streptococci varies and because the method of protective action of the serum is little known. Usually the dose injected is regulated according to cubic centimeters of polyvalent anti-streptococcic sera. The potency of this serum decreases relatively rapidly so that it ought not to be used later than six months after the bleeding of the immunized horse.

Anti-streptococcic serum has been used especially in septicaemia, local infections following traumatism, streptococcic pneumonia, meningitis, rheumatism, erysipelas, pueperal sepsis, scarlet fever, secondary infection complicating tuberculosis, and in fact, in all streptococcus infections.

The therapeutic and prophylactic results obtained by the use of anti-streptococcic serum vary considerably and are apparently dependent on various conditions, such as grade and kind of immunizing serum, day of disease on which injections are made, amount of serum injected, virulence of infecting organism, and determination of the invading organism. From the various reports which have been received from clinicians who have used these sera, there can be no doubt as to the beneficial results which may at times be obtained from its use. In order that good results may be obtained, however, it is essential that it be definitely determined that the streptococcus is responsible for the diseased condition, that a high grade of polyvalent serum be given early

in doses varying from 10 to 40 c. c., and that these injections be repeated at intervals of from four to eight hours.

After injection of anti-streptococcic serum the patient usually rests more quietly for several hours. This, however, need not indicate that recovery from the disease will follow. The therapeutic effects of the serum are manifested by the relief of symptoms, decline in fever, improvement of the pulse, and subsidence of the nervous symptoms. These effects usually appear within twenty-four hours after injection if the serum is to be of value, and if no relief comes within twenty-four hours after two injections of from 20 to 40 c. c. of serum, no beneficial results from the use of the serum are to be hoped for. No untoward effects of the serum are met with except the occasional skin rashes which have already been discussed elsewhere.

Anti-streptococcic serum, even though its curative results are uncertain, ought to be used in every case of acute streptococcus infection. The use of the serum in scarlet fever is not universal but probably should be resorted to in the severer cases. In the chronic cases of discharging sinuses, etc., streptococcuc vaccines are at times used to greater advantage than anti-streptococcic sera.

ANTI-MENINGOCOCCIC SERUM.

Agglutinating substances were discovered in 1903 by Jaeger, in rabbits experimentally immunized to meningococci. Since then numerous attempts have been made to produce specific anti-sera to be used in passive immunization of the human. It was not until 1906, however, that injection of such anti-meningococcic serum was followed with any degree of success. In 1906 Kolle and Wasserman reported results on the use of specific meningococcus serum which they had been able to produce in animals either by intravenous or subcutaneous injections of cultures of meningococci killed by heating to 60° C., or by injections of extracts of meningococci, obtained by shaking these organisms for four or five days in suspension in distilled water. Jochman in this same year reported results on a specific anti-meningococcic serum prepared after similar methods. The effects obtained by the use of these sera have, however, not been as

favorable as those obtained by the injection of a serum prepared by Flexner and Jobling.

The method of preparation of the curative meningococcic serum of Flexner and Jobling, is to inject into the horse first increasing doses of killed meningococci, later increasing doses of living meningococci and finally increasing doses of an extract of meningococci. The extract injected contains the endotoxin liberated by the action of a meningococcus autolytic enzyme which as Flexner has found, is able to destroy the cell substance of the meningococcus.

The action of Flexner and Jobling's serum, according to the originators, is three fold. It exerts an anti-toxic action, is bactericidal and is bacteriotropic or opsonifying. Of these effects clinicians who have used the serum emphasize especially the apparent anti-toxic action.

Flexner and Jobling have summarized the results of the treatment of 400 cases of epidemic cerebrospinal meningitis. According to this summary it has been found by the various clinicians using this serum, that the period of illness can be shortened, the chronic lesions largely prevented and the percentage of mortality markedly reduced. Probably the principle reason for the difference in efficiency of Flexner and Jobling's serum as compared to Kolle and Wasserman's and Jochman's specific sera lies in the method of application. Flexner and Jobling have emphasized the importance of reaching the causal organisms with the serum. For this reason lumbar puncture and withdrawal of a certain amount of cerebrospinal fluid is first made, after which, without removing the needle, injection of the serum is made directly into the sub-arachnoid space of the spinal cord. Recently the persistance of Diplococcus intracellularis in the lateral ventricles, after apparent death of the organism in the spinal sub-arachnoid space, has been emphasized by Knox and Sladen. For the treatment of this condition, Cushing and Sladen have suggested the advisability of ventricular puncture and injection of anti-meningococcus serum.

The results which have been obtained by the use of anti-meningococcus serum as made by Flexner and Jobling, have been so beneficial in acute and chronic cases of meningitis due to the Diplococcus intracellularis that its use is indicated as a specific therapeutic measure.

ANTI-GONOCOCCIC SERUM.

Various attempts have been made to produce an anti-gonococcic serum for passive immunization of man. It is a well known fact that in the human no immunity results from an attack of gonorrhea, and for this reason, together with the lack of favorable results from the use of anti-gonococcic serum, most investigators have concluded that a specific immune serum for Mic. gonorrhoeae cannot be produced. In most of the attempts at the production of anti-gonococcic serum old cultures of the organism have been used.

In 1906, Torrey described an anti-gonococcic serum with which he had attained beneficial results in the treatment of some cases of gonorrheal arthritis. In an attempt to make anti-gonococcic serum, Torrey made the observation that the toxin of the Mic. gonorrhoeae, which is present in old fluid cultures, is toxic for the small laboratory animals. The toxin for the gonococcus has been studied at various times, and has been generally regarded as being derived from the dead and disintigrated bodies of the organism, though by some it has been claimed to be a true extracellular toxin. Torrey found that it is not possible to immunize the small laboratory animals to this toxin, but that in fact at times a true hypersusceptibility may develop as a result of injections of this toxin. This investigator observed, however, that animals can be immunized to the living and dead organisms of gonorrhea, and that in the blood of animals so immunized agglutinins and lysins are present. Based on these observations, Torrey holds that the efficiency of the serum depends upon specific bactericidal substances which act because of lytic rather than of opsonifying power. Phagocytosis, according to this investigator, is of little importance in the destruction of gonococci.

Torrey and Rogers have prepared an anti-gonococcic serum for use in treatment of gonorrheal infections in man. The method of preparation of this serum, as perfected and recommended by these investigators, and used by the different serum producers is as follows: Strong healthy rams receive intraperitoneal injections of increasing amounts of twenty-four hour old ascitic agar cultures of various, recently isolated, virulent strains of Mic. gonorrhoeae. The culture is suspended in salt solution, and for the first two

injections, this suspension is heated to 65° C. for one-half hour before the injection is made. Nine or ten injections are usually necessary to produce serum of high value. After immunization is completed, the animal is bled from the carotid arteries, and the serum allowed to separate out. After this the serum is collected, filtered and tested for sterility. Immunization is made in rams because blood from these animals is apparently little toxic for the human. Polyvalent serum is used because the immune bodies for one strain are specific for that strain, and inasmuch as the strains of gonococci vary in the different infections, immune bodies for all strains must be present in the serum.

Standardization of this serum has not been effectual. It seems quite definite that this serum possesses no anti-toxic nor opsonic value, but is dependent for its efficiency on its lytic action. Because of a lack of any better method of determining the immunizing value of this serum, determinations have usually been made of its agglutinating value. The amount of serum injected is based on cubic centimeters rather than units of immune substance.

Torrey and Rogers' anti-gonococcic serum has been tried in the treatment of the various gonorrheal infections. From time to time favorable and unfavorable results have been reported. The greatest value of the serum apparently is manifested in the treatment of the complications of gonorrheal urethritis, such as prostatitis, epididymitis, orchitis, cystitis, salpingitis, endocarditis, pleuritis, meningitis and especially arthritis. The acute conditions, urethritis, vaginitis, and conjunctivitis, are little effected by this treatment.

The method of treatment with anti-gonococcic serum consists of injections of 2 c. c. of serum at intervals of from one to four days, as may be indicated clinically. The injections of serum are made in the loose subcutaneous tissue in convenient parts of the body. In every case treated with anti-gonococcic serum all the other methods known to be of value in the treatment of the particular condition should be employed. Anti-gonococcic serum seems to cause serum disease more frequently than most of the other immune sera. In all cases it is to be remembered that, because this method of treatment frequently must be carried over a long period of time, serum injections must be made at intervals of not more than seven to eight days.

While anti-gonococcic serum, as prepared by Torrey and Rogers' method, has produced beneficial results in many conditions, it is to be remembered that improvement and recovery have not constantly followed its injection. Moreover injection of this serum ought not to be the only method of treatment instituted.

ANTI-PNEUMOCOCCIC SERUM.

Man after an attack of pneumonia possesses a certain degree of immunity. Immunity, however, does not always follow pneumonia, nor is it lasting. Early in the history of the diplococcus of pneumonia, attempts were made to artificially immunize animals, with the result that it was found that a certain degree of immunity can be produced. In as much as the pneumonococcus does not produce an extracellular toxin, anti-toxic immunity cannot be produced in animals as a result of artificial immunization with this organism.

The anti-pneumococcic serum now used is usually taken from horses that have received increasing injections of dead and later of living cultures of recently isolated pneumococci. Most of the sera are polyvalent, the best known being those made by Roemer and by Pane. The method of action of anti-pneumococcic sera is little understood. It is quite definitely established that it possesses no anti-toxic value. The curative action of the serum then, must depend on its anti-bacterial power. Lysins, until recent times, were supposed to account for the immunizing value of the serum. Neufeld and Rimpau and Wright and others have shown that anti-pneumoccic serum contains bacteriotropic or opsonifying substances which prepare the cocci for ingestion by the leucocytes.

In the treatment of pneumonia with anti-pneumococcic serum usually relatively large amounts of serum are injected. The treatment as recommended by clinicians who have had most experience, is to inject 20 c. c. into the subcutaneous tissue twice a day until the symptoms are relieved. None of the other known methods of treatment are suspended during serum treatment.

The results which have been attained by the use of the serum have varied a great deal. Some observers have lauded the use

of this serum, but when all cases are considered the results are not convincing as far as the curative effects of the serum are concerned. The reasons for the failure of a specific anti-pneumococcic serum are probably dependent on the absence of an anti-toxin in the serum, injection after the disease is far advanced, and the difficulty in determining the species of organism causing the pneumonia. The latter reason must be evident to the clinician, because the disease of lobar pneumonia may be caused by various different species of organisms. Although in general, the therapeutic action of anti-pneumococcic sera has not been marked, still physicians who have used it in pneumonia have been impressed with the general improvement of the patient, the lowering of the temperature and the absence of complications, following its use. Important untoward symptoms seldom have developed from its use.

While anti-pneumococcic serum is not definitely a curative measure, the physician is warranted in using it whenever the patient's condition becomes serious. In all cases it is to be remembered that if beneficial results are to be obtained with this serum it must be injected early.

ANTI-TYPHOID SERUM.

Before the discovery of the organism causing typhoid fever, it was known that an attack of typhoid fever, usually gives some protection against a second attack of the disease. After the discovery of the typhoid bacillus it was found that animals repeatedly injected with non-fatal doses of this organism will ultimately be protected against otherwise fatal doses. It was also found that by injecting blood serum from immunized animals, a passive immunity against typhoid bacilli can be conferred to other animals. Based on these results various anti-typhoid sera have been made for the specific treatment of the disease of typhoid fever in man.

Anti-typhoid sera are usually obtained from horses which have been immunized either by repeated increasing injections of dead and living cultures of typhoid bacilli or typhoid bacillus toxins. Most of the sera prepared have, however, not been anti-toxic but anti-bacterial. Chantemesse has prepared a so-called anti-toxic serum by injecting horses with increasing doses of pure cultures of typhoid bacilli grown for some time on a macerated

splenic pulp and defibrinated human blood medium. Tavel by repeated injections of two weeks old bouillon cultures, sterilized by the addition of 0.5 per cent of carbolic acid has made a serum which is supposed to possess anti-toxic substances. Most of the sera are, however, made by injecting into horses and other animals, increasing doses of dead and living typhoid bacilli of various strains. For these sera little or no anti-toxic value is claimed, the immunizing properties being dependent on the lytic power of the serum.

In the treatment of typhoid fever with these sera, 10 c. c. and frequently larger quantities are injected daily until improvement occurs.

The results following the use of anti-typhoid serum have been disappointing. In most cases no effects on the course of the disease have been observed following the use of this serum. Chantemesse in 1902, reported, that by the use of his serum the death rate in children due to typhoid fever was 3 per cent, whereas the death rate in all the children of Paris who received no such serum injections, was 19 per cent. Occasionally observers have obtained and reported cases in which improvement is rapid after the injection of anti-typhoid serum. As is the case with many of the anti-bacterial sera, there is in general no influence on the course of the disease. In some cases, however, a drop in the fever and improvement in the pulse and general conditions have been reported as resulting from the use of this serum. Various reasons have been assigned for the failure of the curative action of the serum, the most important of which are, the lack of anti-toxic substances which can combine with the toxin liberated after solution and disintegration of the bacilli due to the specific lytic substances in the serum, and, the failure of the body to supply a sufficient amount of complement so as to get the destruction of the invading typhoid bacilli.

Jez has prepared an extract from the spleen, bone marrow and lymph glands of animals immunized to typhoid bacilli. This extract is usually administered by the mouth, and is supposed to possess anti-toxic properties. The value of this extract is doubtful.

Anti-typhoid sera are still in the experimental stage. With the sera now produced no marked beneficial results can be hoped for, and are only used in certain severe cases of the disease.

ANTI-DYSENTERIC SERUM.

Soon after the discovery of the bacillus of dysentery by Shiga in 1892, the treatment of bacillary dysentery by the use of immune sera was undertaken. It was found by Shiga, that the organism which is now regarded as the etiological factor in bacillary dysentery is readily agglutinated with blood from patients suffering with this disease. Following the discovery of the etiological importance of this organism in the dysenteries in Japan this organism was found by Flexner in the dysenteries in the Philippine Islands, and in an epidemic of dysentery in Germany by Kruse. In 1903, Duval and Vedder found this organism in the discharges of dysentery patients in United States, and in 1903, Duval and Bassett, working at the Thomas Wilson Sanitarium near Baltimore, Maryland, isolated this organism from the stools of children suffering with summer diarrhœa. Since Shiga's discovery of the organism, B. dysenteriae has been found in association with dysentery in almost all parts of the world, and the etiology of bacillary dysentery now seems well established. While at first it was supposed that the different organisms isolated from stools in these cases were the same, later investigation has shown that there are two principal varieties of the species of Bacillus dysenteriae. Of these the original Shiga isolation represents a strain which does not ferment mannite, while the organism isolated by Flexner represents a type which produces acid on mannite. The mannite fermenting type has now been found to include at least two and probably three different strains. The original Shiga type has been found especially in the cases of epidemic dysentery in adults, while the mannite fermenting types are most frequently found, although not exclusively or as the only type, in the stools of children suffering with summer diarrhœas. The latter fact emphasizes the especial importance of the mannite fermenting types of dysentery bacilli to the dysenteries in the United States.

A further difference has been found between the two main types; the mannite fermenting types do not produce an extracellular toxin, while the type, not producing acid on mannite, produces an extracellular toxin. Because of these differences in the ability to produce extracellular toxin, antitoxic immunity can be produced by experimental immunization for the Shiga type, while for the mannite fermenting types the production of

anti-toxic immunity is not possible experimentally. These differences have been recognized recently and only after much work had been done on the treatment of bacillary dysentery with specific immune sera. Because of this the value of serum therapy in dysentery can be determined only from the relatively recent literature on this subject.

The results obtained by the use of anti-dysenteric serum in the treatment of dysentery have differed markedly. In Japan, by the use of Shiga's anti-dysenteric serum, the mortality of dysentery has been reduced from 22-26 per cent to 9-12 per cent. Vaillard and Dopter have collected statistics on two hundred cases treated with anti-dysenteric serum, in which cases there was a mortality of only 2 per cent. In the United States on the other hand, no great beneficial results have been attained by the use of the serum as is evidenced from the extensive investigations made in 1903 under the direction of Dr. Flexner.

There are various reasons for the differences in results which have been obtained by the use of anti-dysenteric serum. The patients treated in the United States were largely children under three years of age, while a large percentage of the cases for which favorable results are obtained with serum treatment, occurred in adults. The day of the disease on which serum injections are made is probably earlier in such locations where epidemic dysentery is regarded as a serious disease. In the United States many cases of frequency of stool are only simple diarrhœas, because of this the patient usually receives no medical attention until the disease is well advanced. Probably the most important reason for the difference of the curative value of the different sera is dependent on the differences in the specific substances in the serum. Evidently those sera which have produced the most beneficial results possess anti-toxic properties, while those used in the United States are principally or wholly anti-bacterial. Shiga says of his serum that it is "bactericidal as well as anti-toxic and—therefore is more effective than anti-typhoid serum." Practically all of the anti-dysenteric sera have been polyvalent, i. e., the animals furnishing the serum have been immunized to the various strains and toxins of dysentery bacilli, as the case may be.

The method of treatment with anti-dysenteric serum varies with the severity of the disease. Shiga suggests that in mild

cases one dose of 10 c. c. of the serum be injected. In cases of medium severity two injections of ten c. c. each, the interval between injections ranging from six to ten hours, are recommended. In the severer cases, 40 to 60 c. c. in all are to be injected, but never more than 20 c. c. daily. The serum used in the United States has been injected in larger amounts, sometimes as much as 100 c. c. being injected in one day.

The effect of treatment with Shiga's serum has been to decrease the number of stools, cause the blood and pus to disappear from the stools, restore the temperature to normal, and to lessen the pain and tenesmus. When this serum is used late in the disease the beneficial effects manifest themselves much later. The serum used in the United States has not influenced the course of the disease to any extent, nor is it possible to determine any particular improvement in the condition of patients so treated.

Coyne and Auche have reported eleven cases of dysentery produced by the Flexner, or mannite fermenting, type of dysentery bacillus, which were treated very successfully with a polyvalent serum. The curative effects of this serum were probably due to the action of the anti-toxin to the Shiga type of bacillus dysenteriae.

It is to be hoped that specific anti-toxic sera can be made for the mannite fermenting group of dysentery bacilli, or that Shiga type anti-toxin will be found to be efficient in combatting infections with the mannite fermenting group of dysentery bacilli. This is especially desirable because the mannite fermenting organisms are largely responsible for the childrens' summer diarrhœas in the United States.

ANTI-STAPHYLOCOCCIC SERUM.

Various anti-staphyloccic sera have been made. They are usually obtained from horses and other animals that have received repeated injections of dead and living cultures of these organisms. The method of its action is not clearly understood although any protective value it may have is probably due to lytic and opsonifying power. The value of the serum, as it can now be obtained, is inconsiderable, and its injection in the treatment of staphylococcus infections is seldom or never warranted.

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